Relationships between Carbohydrate Supply and Reserves and the Reproductive Growth of Grapevines (*Vitis vinifera* L.)

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Abstract

Viticultural practices such as trunk girdling and shoot topping along with defoliation, shading and node number per vine treatments were used to alter the carbohydrate physiology of mature Chardonnay grapevines growing in the cool climate of Canterbury, New Zealand.

The timing of vine defoliation in the season previous to fruiting decreased concentrations of over-wintering carbohydrate reserves (mostly starch) in both the trunks and roots of grapevines. Roots were particularly sensitive, with defoliation as early as 4 weeks after bloom in the previous season reducing starch concentrations to 1.5%Dwt at bud burst compared with 17%Dwt in non-defoliated vines. In contrast, partial vine defoliation as early as bloom in the previous season reduced root starch concentrations to 4-7%Dwt at bud burst compared with 15%Dwt in non-defoliated vines. Vine shading and trunk girdling treatments at bloom in the previous season, resulted in small reductions in root starch concentrations (16%Dwt) compared with non-shaded and non-girdled vines (19%Dwt), but shoot topping did not. Study across three growing seasons established that higher concentrations of over-wintering trunk and root carbohydrate reserves were associated with warmer and sunnier weather in the previous growing season.

Individual shoot leaf removal at either the beginning or towards the end of the inflorescence initiation period, reduced shoot starch concentrations to 3-6%Dwt compared with 11%Dwt for no leaf removal, such reductions persisted through to the following season. Shoot topping at the start of the initiation period had no effect on shoot carbohydrate accumulation, but trunk girdling temporarily increased shoot starch concentrations during the first 31 days after treatment.

Reductions in over-wintering trunk and root carbohydrate reserves were associated with a reduction in inflorescences per shoot and flowers per inflorescence in the following season, the reduction as much as 50% compared with non carbohydrate stressed vines. While there were strong linear or curvilinear relationships between the concentration of starch in trunks and roots at bud burst and inflorescences per shoot and flowers per inflorescence, in case the of inflorescences per shoot, there was not an immediate cause and effect because inflorescences were initiated in the previous season. Individual shoot leaf removal during the inflorescence initiation period illustrated that leaf removal directly inhibited the initiation of inflorescences in latent buds. Shoot carbohydrate

measurements showed a strong curvilinear relationship to the number of inflorescences per shoot, with a threshold starch concentration of 10-12%Dwt during the inflorescence initiation period required for a maximum number of inflorescences per shoot. Furthermore, examination of individual node positions emphasised the importance of the subtending leaf on the initiation of inflorescences within the latent bud.

The number of inflorescences per shoot post bud burst was reduced on vines that were both carbohydrate reserve stressed (by previous season's defoliation) and had a high node (108) number retained per vine after winter pruning compared with little or no reduction in inflorescences per shoot on carbohydrate reserve stressed vines that had a low (20) node number per vine. The reduction in inflorescences per shoot on high node vines was associated with reduced carbohydrate reserves and reduced shoot vigour (thinner and lighter shoots).

Flowers per inflorescence were reduced by as much 50% in response to lower over-wintering carbohydrate reserves. Fewer flowers per inflorescence were attributed to a reduction in primary branching of the inflorescence and also a reduction in flowers per branch. Strong linear relationships between the concentrations of starch in trunks and roots and flowers per inflorescence indicate that the determination of flowers per inflorescence, unlike inflorescences per shoot, may be dependent on the level of over-wintering carbohydrate reserves. This is most likely due to changes in branching of the inflorescence and individual flower formation occurring during the bud burst period. Per cent fruitset was not affected by reductions in carbohydrate reserves, so fewer inflorescences per shoot and flowers per inflorescence resulted in reduced vine yield.

The findings of this thesis indicate that changes in the level of carbohydrate production and partitioning in response to a range of viticultural management practices and seasonal weather contribute to seasonal variation in grapevine flowering and yields in New Zealand's cool climate environment. The relationships between carbohydrate reserves and flowering illustrate the potential to use this information to predict grapevine flowering and forecast yields. The practical implications of this research illustrate that the viticulturist must manage grapevines not only for the current crop, but also for subsequent crops by maintaining sufficient carbohydrate reserves for balanced growth, flowering and fruiting from season to season.

Key words: over-wintering carbohydrate reserves, starch concentrations, roots, trunks, shoots, defoliation, girdling, topping, inflorescences, flowers, fruitset, yield.

Contents

All line drawings and colour plates unless otherwise stated were produced and/or photographed by the author.

List of Tables

List of Figures

List of Plates

List of Appendices

List of Abbreviations

μM	micromolar
μmol/L	micromoles per litre
nm	nano metres
mm	millimetre
cm	centimetre
m	metre
defol.	defoliation
no defol.	no defoliation
Dwt	dry weight
mg	milligram
g	gram
kg	kilogram
kPa	kilopascal
lsd	least significant difference
mL	millilitre
NS	not significant
°C	degrees Celsius
rpm	revolutions per minute
DABB	days after bud burst
EBSN	early bunch stem necrosis
BN	bud necrosis
CHO	carbohydrate
VSP	vertical shoot positioning

Chapter 1

General Introduction

Wine grapes have been grown throughout New Zealand for over 100 years, but significant industry success has only occurred in the last decade since the flourishing export trade of New Zealand wines has developed. In 1991 a total of 5440 hectares of plantings were recorded, by 2001 that had more than doubled to 11650 hectares. However, although production area has more than doubled total grape production has increased little (64000T in 1991 to 69000T in 2001). Despite this export earnings have increased from $27million in 1991 to $198million in 2001 and now account for 34% of New Zealand's wine sales. New Zealand wine production, however, only represents 0.2% of global production (New Zealand Horticulture Facts and Figures 2001). The near static growth in grape production can be attributed to a general decrease in average yield over the last 15 years (Figure 1.1a). The reduction in average yield reflects the change from high yielding varieties to lower yielding premium varieties. The production area of Müller Thurgau (high yielding, 11.2-16.6T/ha) has steadily decreased over the last 13 years, while the production area of lower yielding Chardonnay and Sauvignon blanc varieties (5.2-9.4T/ha) has consistently increased (Figure 1.1b) (New Zealand Horticulture Facts and Figures 2001). Plantings of premium red varieties such a Pinot noir and Merlot have also increased over this period (New Zealand Horticulture Facts and Figures 2001).

Along with the decreasing trend in average yields over the last 15 years, distinct variation in yield from season to season is also evident (Figure 1.1a). Seasonal variation in yields has been reported to cause inefficiencies in the wine production process, influencing

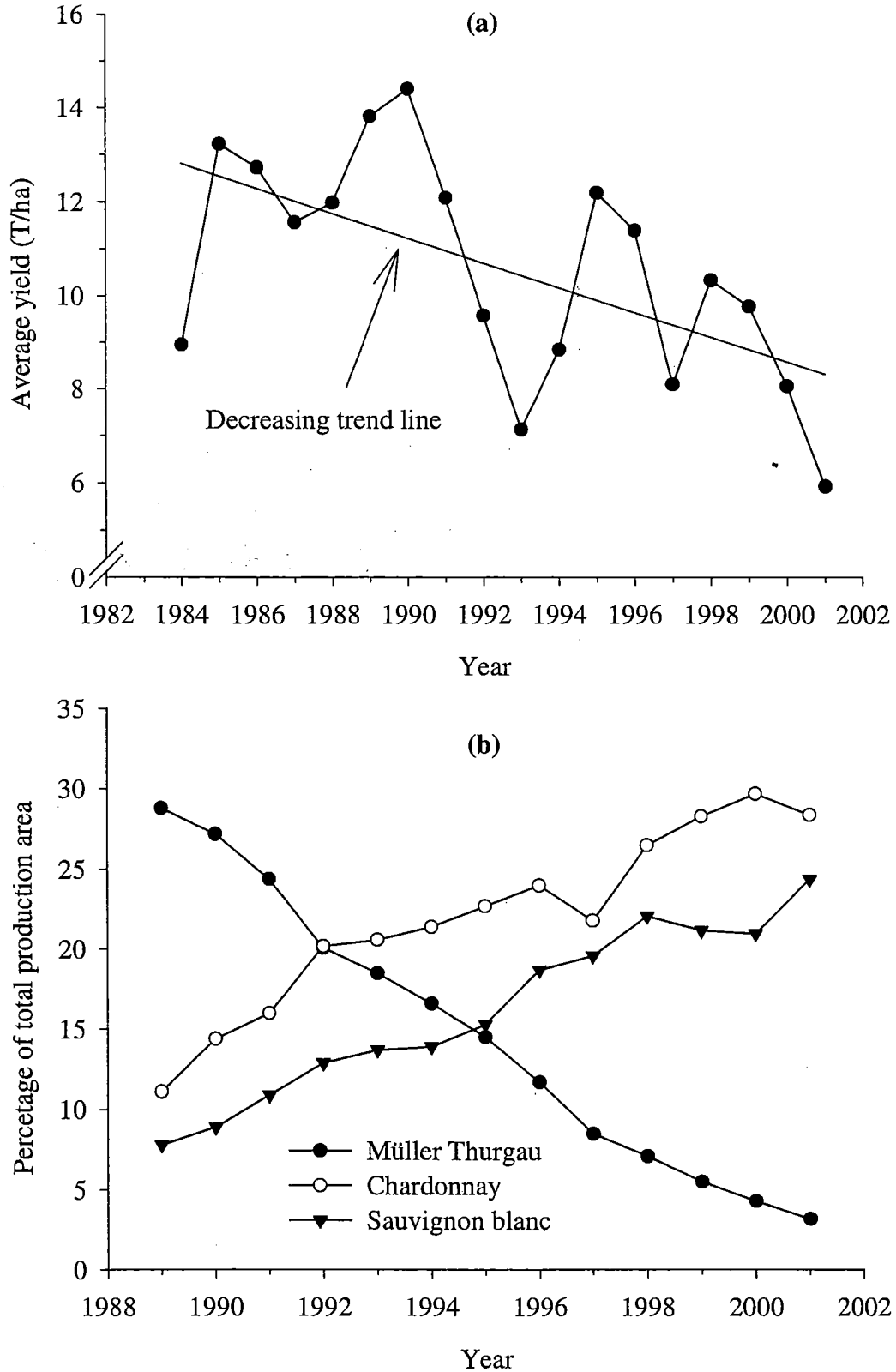

Figure 1.1 Average yield per hectare (a) and percentage area of production by the three main vinifera cultivars (b) over the last 13 years in New Zealand. Adapted from Trought (2001).

processing capacity, wine storage and marketing resulting in foregone revenue and extra costs (Martin *et al.* 2000). Recognising this, winery and vineyard managers endeavour to forecast yield each season. Yield forecasting methods are based on the measurement of a range of yield components, which include: per cent bud burst, inflorescences or clusters per shoot, flowers per inflorescence, percentage fruitset, berries per cluster and berry and cluster weights (Martin *et al.* 2000, Wilson 1996). Either individual vines or transects of canopy (unit length of row) can be measured in the vineyard (Dunn and Martin 1998, Martin *et al.* 2000, Folwell *et al.* 1994). However, even experienced vineyard managers who have worked on the same vineyard for decades continue to find it difficult to forecast yields accurately. Surveys in Victoria, Australia, indicate an average forecasting error of approximately ± 25% (Martin *et al.* 2000). Such a large error level indicates that yield forecasting methods need to be refined. Part of the refinement process could include obtaining detailed information on vine physiology, for example, the level of over-wintering carbohydrates (CHO) reserves and their relationship with vine flowering and subsequent yield. Such information may assist to better integrate the seasonal variation influence that exists, particularly in cool climate environments like that of New Zealand.

However, before such refinement is possible a much greater understanding of the influence of vineyard management and environmental factors on yield components is necessary, in particular, the effect on physiological processes that influence vine flowering and fruiting. Seasonal variations in New Zealand's average grape yield (Figure 1.1a) suggest that seasonal weather conditions have a strong influence on yield. There is however, very little documented data to illustrate which components of yield are the cause of the yield variation in New Zealand. Preliminary studies by Mac Gregor (2000) in California and Trought (2001) in New Zealand suggest that temperature and accumulated heat (growing degree days) in the season previous to fruiting strongly correlate with inflorescence number per shoot and yield. It is proposed that the positive influence of temperature and accumulated heat on following season's yield is mediated by the effect that these climatic factors have on the production and partitioning of photosynthates to reserve organs and hence the level of over-wintering CHO reserves stored in trunks and roots. It has been previously suggested by Hunter *et al.* (1995), Koblet (1996) and Sommer *et al.* (2000) that the productivity of grapevines in cool

climates, in particular, is a function of CHO reserves stored in the permanent woody parts of the vine (mostly trunks and roots), yet none of these authors have established the physiological relationships between CHO and flowering and fruiting in grapevines.

The research presented in this thesis therefore seeks to investigate, in detail, the relationship between carbohydrate physiology of the grapevine and both floral development and yield realisation in a cool climate environment. The aim of these investigations is to gain a better understanding of the role of CHO physiology in the flowering of grapevines and to determine whether there is a potential to use physiological information to improve the forecasting of grapevine flowering and yields.

The experimental content of the thesis research is divided into four chapters which cover the systematic investigation of the influence of a range of viticultural practices on CHO reserve accumulation in grapevine shoots, trunks and roots and following season's vine flowering and productivity. These include;

- The influence of the intensity of vine defoliation on yield components and yield realisation in the following season (Chapter 4). It is hypothesised that increasing intensity of vine defoliation results in a greater reduction in floral components of yield (inflorescences per shoot, flowers per inflorescence and fruitset) in the following season.

- The effects of the timing of vine defoliation, shading and seasonal variation on CHO reserve accumulation in the trunks and roots of grapevines, and the relationship between over-wintering CHO reserves and floral components of yield (Chapter 5). Three hypotheses are investigated. Firstly, that vine defoliation and shading reduces the level of over-wintering CHO reserves accumulated in both trunks and roots. Secondly, that reduced levels of over-wintering CHO reserves in trunks and roots restrict the development of floral components of yield (inflorescences per shoot, flowers per inflorescence and fruitset). Thirdly, that inflorescences per shoot has more influence on vine yield than flowers per inflorescence and per cent fruitset.

- The effects of timing of individual shoot and vine defoliation on the accumulation of CHO reserves in shoots, trunks and roots and the initiation of inflorescences and yield realisation in the following season (Chapter 6). It is hypothesised that defoliation not only reduces photosynthate supply to CHO reserve recharging, but also to the inflorescence initiation process in latent buds. The effects of defoliation on reduced inflorescence number per shoot is therefore hypothesised to be a consequence of reduced CHO supply to latent buds and CHO accumulation in shoots during the period of inflorescence initiation.

- The influence of trunk girdling and shoot topping on the accumulation of CHO reserves in shoots, trunks and roots and the initiation of inflorescences and yield realisation in the following season (Chapter 7). Two hypotheses are investigated. Firstly, that trunk girdling and shoot topping increase shoot CHO accumulation and the supply of CHO to latent buds with the consequence that the number of initiated inflorescences per shoot is increased. Secondly, that trunk girdling reduces the accumulation of root CHO reserves, which in turn impacts negatively on the flowering, yield and growth of grapevines in the following season.

Chapter 2

Review of the literature

2.1 Grapevines as a horticultural crop

In the wild, *Vitis vinifera* L. is a vigorous climbing plant of deciduous forests and its trunk and branches are flexible with the vine being supported by the trees on which it grows. The climbing habit of the grapevine is exemplified by the occurrence of tendrils, which enable the vine to climb 20-30m into a forest canopy. Grapevine cultivation in the European tradition is based on either small free standing bushes or the training of vines onto supports. In modern mechanised viticulture grapevines are usually trained onto post and wire trellises of many different designs. The grapevine in its wild state produces large numbers of small clusters of fruit, however as a crop plant the grapevine is severely pruned to reduce cluster number, increase fruit size and fruit quality. The grapevine has a remarkable ability to regrow after pruning, which enables the easy renewal of annual wood for fruiting in the following season. Carefully tended grapevines remain productive for a very long time, however in commercial viticulture, grapevines are seldom retained for more than 40 years (Mullins *et al.* 1992).

The wine grape *Vitis vinifera* L. is a temperate climate species, which cannot withstand extreme winter cold and requires warm to hot summers for the maturation of its fruit. This requirement limits its cultivation, in general, to latitudes situated between 30°N and 50°N and between 30°S and 40°S. New Zealand has the world's most southerly plantings of grapevines at a latitude of 45°S (Jackson and Schuster 1994). Along with vinifera there are about sixty species of *Vitis*, many of which have been intercrossed to produce

cultivars with increased cold and disease tolerance. However, in general modern day viticulture is still based on a range of both red and white classical grape varieties that were selected over the centuries of cultivation in Europe, before their introduction into New World viticulture. Today's viticulture is primarily based on the use of grafted plants in which rootstock, either a North American species or interspecific hybrid, is resistant to soil-borne pests such as phylloxera and nematodes (Mullins *et al.* 1992). A wide variety of rootstocks are now available to suit a range of climates, soil types and production methods.

Viticulture in New Zealand is primarily based on the use of the Vertical Shoot Position (VSP) training system using either canes or spurs (Figure 2.1). VSP cane pruned vines consist of a single trunk which terminates in a head at the fruiting wire. From the head canes are laid down each winter to fruit in the following season (Figure 2.1a). VSP cordon pruned vines consist of a single trunk with cordons that are permanently attached to the fruiting wire. Annual shoot growth is pruned back to spurs, which will fruit in the following season (Figure 2.1b). In New Zealand's cool climate the VSP training systems allow for easier disease control and improved fruit maturation (Jackson and Schuster 1994).

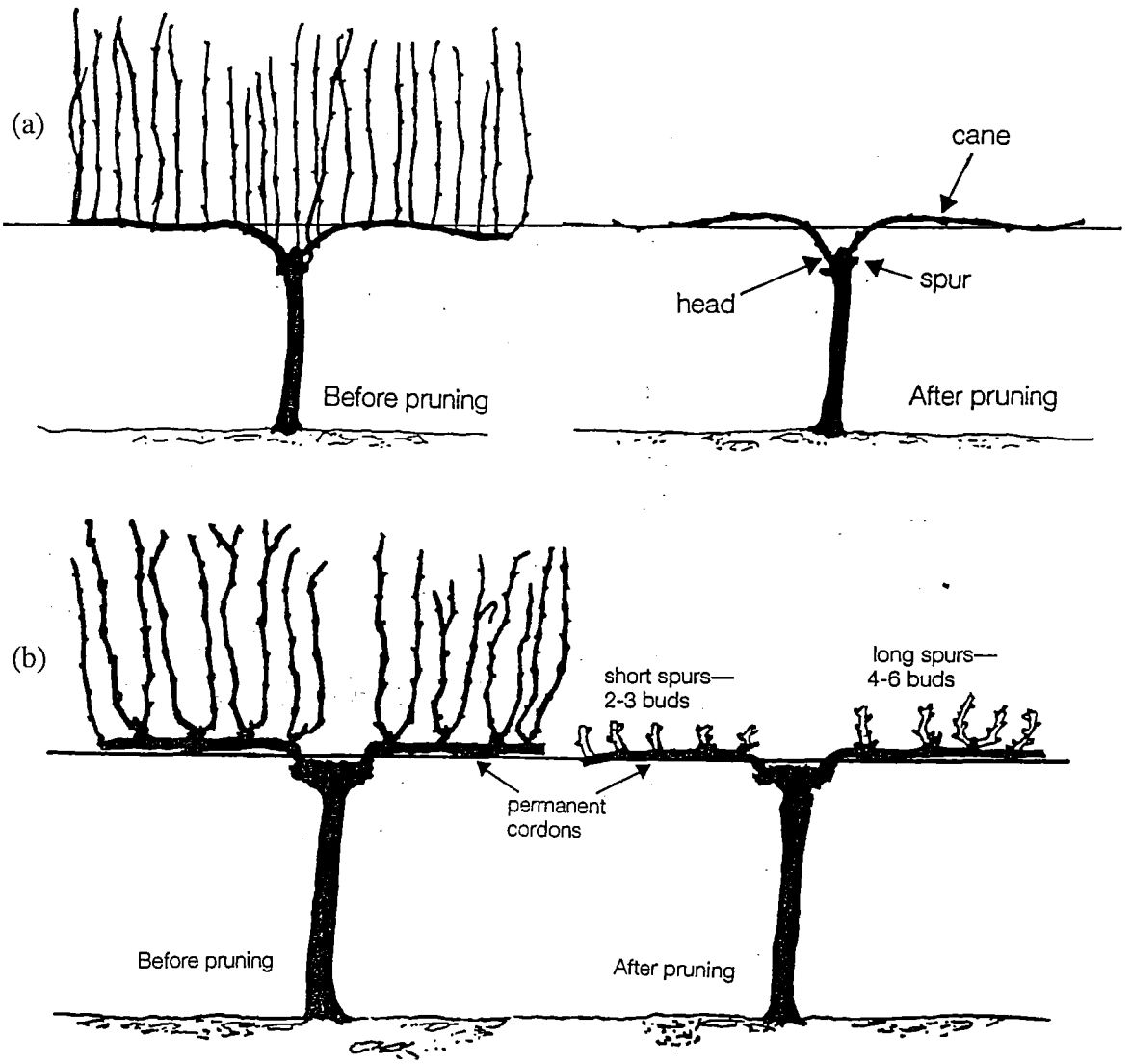

Figure 2.1 Vertical shoot position (VSP) training systems, (a) cane pruned and (b) cordon spur pruned (adapted from Jackson and Schuster 1994).

2.2 Grapevine phenology

A good understanding of grapevine phenology throughout the growing season is vital in order for any viticulturist to obtain the optimum performance from their grapevines. The following phenological stages are based on the modified system of Eichhorn and Lorenz (E-L) developed by Coombe (1995) (Figure 2.2).

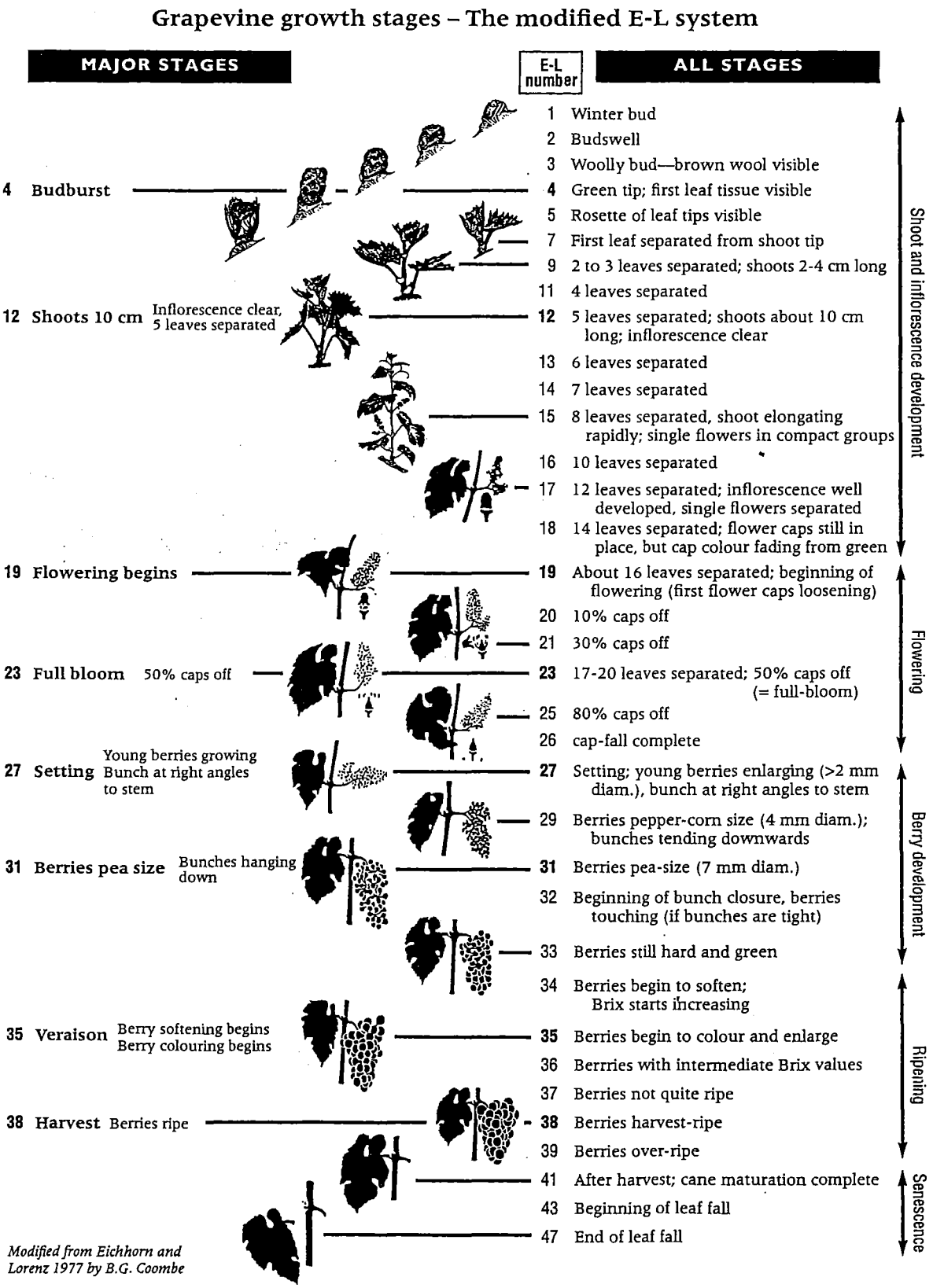

Figure 2.2 The modified E-L system (from Coombe 1995).

Bud burst (modified E-L stages 2-4) occurs when the daily mean temperature exceeds 10°C after enforced dormancy is broken (Moncur *et al.* 1989) and relies on the supply of stored reserves in the canes, trunk and roots (mainly carbohydrates) (Mullins *et al.*1992). The proportion of buds per vine that burst is influenced by the number of nodes (buds) present. May and Bessis (1985) have shown that the proportion of buds that burst decreases with increasing node number per vine.

Shoot and inflorescence development for the first 8-10 weeks following bud break (modified E-L stages 5-18) is rapid. Shoots develop in the first instance from the primary bud of the compound bud. If the primary shoot is damaged or destroyed, for example by spring frost, the secondary or tertiary bud may grow (see 2.4.2) (Jackson and Schuster 1994). Secondary or tertiary buds may also develop shoots when vines are pruned hard or when the parent vine exhibits high vigour. However, both the secondary and tertiary buds are considerably less fruitful (Mullins *et al.* 1992). Initial shoot growth results from an increase in length due to the elongation of preformed nodes (formed in the previous summer) and is referred to as 'fixed growth'. After approximately twelve nodes 'free growth' occurs and is the result of the production of new leaf primordia and nodes by the apical meristem of the shoot in the current season (Mullins *et al.* 1992).

Initial shoot growth is reliant on the supply of reserves (mainly CHO) stored within the cane and permanent parts of the vine until shoots have developed sufficient leaf area to support growth through photosynthesis (McArtney 1998). Once leaves have reached 50% of their final size they become net exporters of photosynthates, which is usually between 20 and 30 days after they first emerge from the shoot tip (Hale and Weaver 1962). As shoot growth develops one to three inflorescences appear usually from node four onwards opposite a leaf (modified E-L stage 12). At higher node positions tendrils develop in place of inflorescences (Mullins *et al.* 1992). The flowers of the inflorescence differentiate and develop very rapidly following bud burst and all flower parts are formed within 10-15 days of the appearance of the inflorescence (Agaoglu 1971), but are not clearly visible until shoots have eight leaves (modified E-L stage 15).

Flowering (referred to as bloom or anthesis) and fruitset (modified E-L stages 19-27) are perhaps the most important times in the development of a grape crop. In terms of vine

phenology, anthesis remains in phase with the formation of leaves and nodes (Coombe 1972), typically 16 leaves and nodes per shoot are formed when anthesis starts (modified E-L stage 19). The grape inflorescence has many flowers each capable, given the right circumstances, of forming a berry, however, typically only 20-30% of the flowers develop into berries, thus many flowers fail to set fruit and potential yields are reduced (Trought 1998). Most viticulturists attribute poor set to bad weather conditions during flowering. While cold, wet weather is a contributing factor, the actual mechanism(s) for the failure of fruitset are not clear but recent investigations point towards photosynthate supply (Caspari *et al.* 1998, Ferree *et al.* 2001, Keller and Koblet 1994). The involvement of CHO reserves in the fruitset process remains unknown at this time. Fruitset is generally completed within 2 to 3 weeks of anthesis (modified E-L stage 27) and fruitlets retained will normally develop to maturity. Vine stress at anthesis/fruitset is known to induce the disorder, Early Bunch Stem Necrosis (EBSN), where parts or whole branches of the inflorescence die and abscise, and consequently large crop losses can occur. Insufficient CHO and/or an imbalance between CHO and nitrogen are implicated as the physiological cause of this disorder (Jackson and Coombe 1988, Gu *et al.* 1994, Keller and Koblet 1995a).

Following fruitset the growth of each berry follows a double sigmoid pattern, which is characterised by three stages. Stage I immediately follows fruitset, fruitlets rapidly enlarge into small hard green berries. Stage II of berry growth is characterised by a period of slow berry growth, during which seed maturation begins. The onset of stage III is marked by berry softening and colour change - referred to as véraison (modified E-L stage 34). Rapid berry expansion resumes and significant juice composition changes occur with decreases in organic acid content and accumulation of hexose sugars (Mullins *et al.* 1992). The fruit is harvested from the vine (modified E-L stage 38) once berry sugars, colour, acidity and flavour have reached a desired level deemed appropriate for the wine style to be made. During stage III of berry growth the cane maturation phase begins, green soft shoots change to yellow and then brown as they become lignified. Depending on the climate the vine may, or may not, remain in green leaf for several weeks following harvest before entering senescence when leaves change to yellow or red in colour before abscising (leaf fall, modified E-L stage 43). After leaf fall the vine enters winter dormancy and will not resume growth until the following spring.

2.3 Grapevine carbohydrate reserves

The grapevine stores carbohydrate reserves in two main forms; starch and soluble sugars (sucrose, glucose, fructose and *myo*-inositol) (McArtney 1998). Small amounts of other sugars such as raffinose and stachyose are found, but contribute little to the carbohydrate reserve pool of the grapevine (Wayne *et al.* 1990, Hamman *et al.* 1996). The concentrations of starch and soluble sugar within the woody tissue of the grapevine vary greatly over the growing season depending on vine phenology. In all aerial parts of the vine (canes, cordons and trunk) starch reserves are at their highest concentration at the end of the growing season. As winter begins starch concentrations start to decrease while soluble sugar concentrations begin to increase (Winkler and Williams 1945, Williams 1996). These changes are associated with the development of winter hardiness, where increases in the concentration of soluble sugars from the conversion of starch act as a cryoprotectant against cold temperature injury (Hamman *et al.* 1996). As the end of the winter approaches the conversion of carbohydrates is reversed and hence starch increases and soluble sugar decreases (Korkas *et al.* 1994a, Winkler and Williams 1945, Williams 1996). Some loss of CHO does occur over the winter as a result of respiration (Williams 1996), due to enzymatic activity during the CHO conversion process. In root wood there is no interconversion of starch and soluble sugar during the winter (Winkler and Williams 1945).

Both in woody fruit trees and vines the root system is often found to have higher concentrations of carbohydrates than any other portion of the tree or vine and therefore is often considered the organ of carbohydrate storage (Loescher *et al.* 1990). Literature from various viticultural regions around the world has illustrated that a wide range of environmental and management factors during the growing season influence carbohydrate reserves in above ground portions of the vine, for example, seasonal conditions (Bains *et al.* 1981, Winkler and Williams 1945), crop load (Balasubrahmanyam *et al.* 1978, Weaver and McCune 1960), leaf area/defoliation (Candolfi-Vasconcelos and Koblet 1990, Koblet *et al.* 1993), vine nutrition (Korkas *et al.* 1996a and b), pests and diseases (Rühl and Clingeleffer 1993, Ryan *et al.* 2000), irrigation (Rühl and Alleweldt 1990) and vine pruning/training (Koblet *et al.* 1993, Schultz *et al.* 2000, Williams 1996) However, most of this literature has only reported

on CHO reserves in canes, cordons and trunks. Less information is available on the carbohydrate physiology of grapevine roots, despite their importance as a primary CHO reserve organ. Little is known of the influence of environmental factors and vine management on root CHO reserves and the consequential effects on vine development in the following season.

There is no doubt that CHO reserves are derived from the products of photosynthesis in leaves (Oliveira and Priestly 1988). Scholefield *et al.* (1978) have shown using ^{14}C - CO_2 that sugars produced in leaves are converted into starch in root wood and that the last sugars converted to starch are the first to be utilised in following season's new growth. Other work (Koblet and Perret 1980, 1982, Korkas *et al.* 1994b, Murisier and Aerny 1994, Yang and Hori 1979, 1980, Yang *et al.* 1980) has also demonstrated that trunks and roots are the organs of CHO reserve accumulation and storage and that these CHO reserves are utilised in the development of new seasons' shoots and inflorescences in the following spring.

The remobilisation of CHO reserves (mostly starch) in the spring is facilitated by the enzymatic breakdown of starch into soluble sugars and can be observed by xylem fluxes of sucrose, glucose and fructose, with glucose predominating (Stoev *et al.* 1959, McArtney 1998). Newly developing shoots are solely dependent on the supply of mobilised CHO reserves until the first few leaves on a shoot become net exporters of photosynthates (Hale and Weaver 1962, Koblet 1969). However, the importation of root CHO reserves into shoots does not cease until around flowering time when shoots have at least 10-16 leaves (Stoev *et al.* 1959, Yang *et al.* 1980). This observation is consistent with the fact that root starch concentrations reach their lowest levels at flowering (Winkler and Williams 1945, Williams 1996). Because root sourced CHO is still transported to developing shoots at the time of anthesis, the possibility that root CHO reserves could be utilised in the fruitset process may exist. However as discussed in 2.2 there is no research at this time to support or refute such a suggestion.

2.4 Latent bud, inflorescence and flower developmental morphology

2.4.1 The reproductive cycle of the grapevine

Before a detailed review of latent bud, inflorescence and flower development is discussed it is important to first understand the reproductive cycle of the grapevine. Understanding when inflorescences and flowers are formed during the growing season is essential for good crop management. This is because vineyard environmental and management factors that affect their development will ultimately alter yield potential and economic viability of the grape crop that follows. The reproductive cycle of the grapevine occurs over a 15 to 18 month period (Figure 2.3). During the first season floral induction in late spring (November-December, Southern Hemisphere) is followed by inflorescence initiation in summer (December-February). By the end of the summer inflorescence primordia become dormant in readiness for winter (May-August). In spring (September-October) of the second season the buds burst into growth and individual flowers are formed on inflorescences. This is followed by flowering and fruitset in early summer (December). Flowers that successfully set develop into berries, which continue to grow throughout the summer (January-February) before ripening in autumn (March-May).

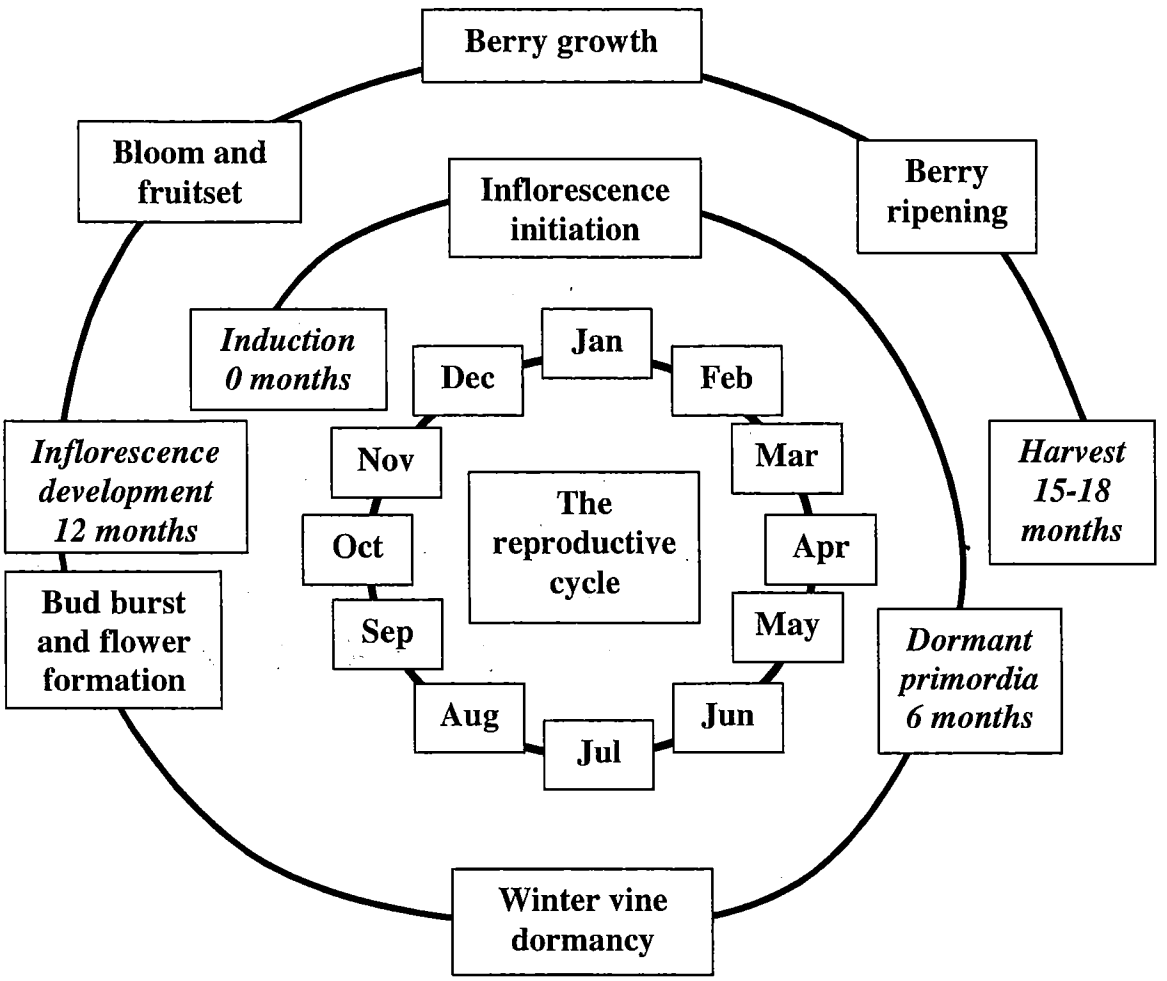

Figure 2.3 The reproductive cycle of the grapevine *Vitis vinifera* L. in New Zealand Adapted from Wilson (1996).

2.4.2 Latent bud and inflorescence development

The bud that is observed to develop in a leaf axil during the summer is referred to as the compound latent bud and is the site where next season's shoots, leaves and inflorescences are initiated (Pratt 1974). Although the compound latent bud appears as an appendage of a summer lateral shoot (Carolus 1970), its association with the main shoot is very close. The xylem vessels of the compound latent bud lead directly to the xylem of the main shoot (Pratt 1974). The compound latent bud is comprised of three buds referred to as the primary, secondary and tertiary buds. Prior to either leaf or inflorescence primordia initiation in the primary bud, the apex of the primary bud first produces two or more

bracts and, in the axils of these, forms the secondary and tertiary buds (Srinivasan and Mullins 1981a). Depending upon the grape variety, the primary bud produces between six and ten leaf primordia and up to four inflorescence primordia (Buttrose 1969a). The secondary bud usually bears one or more inflorescence primordia, although fewer than the primary bud (Pratt 1974) and the tertiary bud is considered vegetative (Srinivasan and Mullins 1981a). The three buds remain enclosed by several bracts and constitute the compound latent bud of the main shoot (Pratt 1974). Fully mature compound latent buds containing one or more inflorescence primordia are referred to as fruitful or fertile buds (Srinivasan and Mullins 1981a). The number of inflorescence primordia formed in each primary bud increases along the main shoot up to approximately node twelve and then decreases beyond (May 1987, Winkler and Shemsettin 1937). These differences between node positions vary in magnitude according to grape variety (Lavee *et al.* 1967, May 1987, May 2000). The increase in the number of inflorescences per primary bud from the proximal to the middle position of the main shoot and then subsequent decrease towards the distal end is thought to be a genetically fixed characteristic (May 1966), but this pattern may be altered by climatic conditions (Buttrose 1974a) and main shoot orientation (May 1966). A detailed review of the developmental morphology and anatomy of inflorescences and flowers is provided by Gerrath (1992).

Floral development in the grapevine involves three well defined phases that are widely recognized (Barnard 1932, Barnard and Thomas 1933, Gerrath 1992, May 2000, Srinivasan and Mullins 1976). The first phase involves the formation of uncommitted primordia called anlagen. Anlagen are club shaped meristematic protuberances which arise from the apices of the primary bud, which may be directed to form inflorescence, tendril or shoot primordia. The second phase involves the conversion of anlagen into inflorescence primordia. During the conversion anlagen undergo repeated branching to form a conical structure composed of many rounded protuberances (inflorescence branch primordia). The third and final phase involves the formation of flowers. Inflorescence branch primordia undergo differentiation to form individual flowers. The first two phases are completed in the season prior to fruiting (Figure 2.3). The final phase occurs shortly before, and during bud burst in the spring of the fruiting season (Figure 2.3, see also 2.4.3).

Following the work of Srinivasan (1978) a phenological code was constructed to describe in detail inflorescence primordia initiation (phases one and two) and flower development (phase 3), which involves twelve stages (0-11) that relate to systemic changes in the shape of organs and the formation of new structures. The formation of a leaf primordia in the newly developed primary bud is the first step in bud development (stage 0). Leaf primordia arise from the apical meristem in acropetal succession with distichous phyllotaxy (Srinivasan and Mullins 1976). Depending on the grape variety the primary bud apex produces three to eight leaf primordia before the first anlage is produced (stage 1). The formation of anlagen from the apex is regarded as the first step of the inflorescence initiation process (Srinivasan and Mullins 1976). Anlagen develop as broad, blunt, obovate structures (stage 2) and are quite different to the narrow and pointed leaf primordia. Further development of the anlagen starts with bract formation (stage 3). The anlagen then divide into arms (stage 4) (Pratt 1971). Inflorescence primordia are formed by extensive branching of anlagen (stage 5). The inner arm divides and gives rise to the main body of the inflorescence (Scholefield and Ward 1975). The outer arm gives rise to the lowest and largest branch of the inflorescence (referred to as the shoulder). Continued branching of the inner and outer arms give rise to branch primordia of the second and third order (stages 6 and 7) respectively. After the formation of one to three inflorescence primordia the primary bud becomes dormant (Pratt 1971).

The inflorescence initiation process occurs over a reasonably short period of time for each primary bud. In Chenin blanc vines, anlagen appear in the basal primary buds of the main shoot from 15 days before anthesis, when approximately twelve expanded leaves are present on the main shoot. The differentiation of anlagen into inflorescence primordia occurs 14 to 21 days after the anlagen first appear and coincides with current anthesis (Swanepoel and Archer 1988). However, Pratt (1979) working with Concord vines notes that the first recognisable inflorescence primordia may not appear until the start of anthesis when the main shoot has approximately thirteen expanded leaves. Once the first inflorescence primordium has been initiated the second begins to develop and once complete the third and fourth inflorescence primordia may develop. The initiation of inflorescence primordia in primary buds at higher node positions on the main shoot progresses in a sequential manner as further shoot elongation takes place and new nodes and leaves appear (Buttrose 1974a, May 1987, 2000).

2.4.3 Flower development

The differentiation of flowers from inflorescence branch primordia (phase three) begins after the primary bud has broken dormancy in the spring of the season following inflorescence initiation (Agaoglu 1971, Barnard and Thomas 1933, Scholefield and Ward 1975, Snyder 1933a and b, Srinivasan 1978, Winkler and Shemsettin 1937) (see Figure 2.3). Agaoglu (1971) suggests that all flowers and flower parts are formed within 10-15 days of the appearance of the inflorescence from the bursting latent bud. Each branch primordium of the inflorescence primordium divides many times and ultimately produces the flower initials (stage 8 of Srinivasan's code) (Srinivasan 1978). Initiation and development of flower parts occurs in an orderly fashion, beginning with sepals and then followed by petals (calyptrae) (stage 9), stamens and pistils (Pratt 1971, Okamoto *et al.* 1977, Considine and Knox 1979) (stage 10). Finally the flower is fully developed in readiness for anthesis (stage 11).

The arrangement of flowers on an inflorescence becomes visually clearer once the inflorescence begins to rapidly elongate prior to anthesis. The number of flowers on the outer arm (shoulder) can be up to one third of that on the inner arm (May 1987). Troll (1964) describes an inflorescence as consisting of a 'main axis' (equivalent to an arm) which terminates in the 'primary florescence' on which flowers are situated singly or in the form of a 'dichasium', a group of three flowers with two placed laterally at the base of the central flower (May and Antcliff 1973, Okamoto *et al.* 1977). The main axis of the inflorescence carries side branches called 'paraclades' (equivalent to branches). The side branches are themselves copies of the main axis and terminate in the same single or dichasium arrangement of flowers and are termed 'coflorescences'. The paraclades can also carry second order paraclades carrying flowers in a single or dichasium arrangement. Similar inflorescence structure and flower arrangement is also observed in *Vitis riparia* (Gerrath and Posluszny 1988).

2.5 Environmental factors influencing inflorescence and flower development

2.5.1 Temperature

There are many reports detailing a requirement for high temperatures for inflorescence primordia initiation in grapes (Baldwin 1964, Buttrose 1974a, Srinivasan and Mullins 1979, Palma and Jackson 1981). Specifically, high temperatures during stages 5 to 7 of inflorescence initiation (Srinivasan 1978) have been shown to correlate well with subsequent fruitfulness of latent buds (Srinivasan and Mullins 1979). Buttrose (1969a) found the effect of high temperature on increased bud fruitfulness was greatest when the node carrying that latent bud was at the main shoot apex at the time of temperature treatment. Palma and Jackson (1981), however, found that high temperature had most influence on inflorescence initiation when the latent bud was three nodes back from the apex of the main shoot at the time of temperature treatment. Both Buttrose (1969a) and Palma and Jackson (1981) suggest that the effect of temperature on inflorescence initiation occurs well before any examinable inflorescence primordia occur within the developing latent bud. Buttrose (1969b, 1970) further concludes that the critical period for susceptibility to high temperature is restricted to three weeks before the formation of anlagen in latent bud apices, when a pulse of only four hours per day or night of high temperature (30°C) is sufficient to induce the successful initiation of a second and third inflorescence primordia.

Some varietal differences have been found in the temperature requirements for inflorescence initiation. Riesling and Shiraz initiate inflorescences at temperatures as low as 20°C, but Muscat of Alexandria requires a temperature of 25°C (Buttrose 1970, Okamoto *et al.* 1977). Sultana and Ohanez grape varieties tend to be less fruitful than other varieties and show more response to changes in temperature (Buttrose 1970). There is little understanding how higher temperatures stimulate inflorescence initiation. One may hypothesise that higher temperatures increase cell division and differentiation of anlagen such that they are directed to form inflorescence primordia rather than tendril or shoot primordia. This in turn may be facilitated by the influence that higher

temperatures might have on increasing the supply of nutrients to differentiating anlagen, for example, the supply of carbohydrates, hormones or minerals.

Temperature is also suggested to play an important role in the development of flowers during and after bud burst in the season of fruiting. Buttrose and Hale (1973) have shown with four vinifera cultivars (Cabernet sauvignon, Shiraz, Rhine Riesling and Clare Riesling) that the rate of inflorescence development significantly increased as temperatures rose, consequently the time taken to reach anthesis was reduced. Under a day/night temperature environment of 14/9°C it took between 65 and 75 days to reach anthesis. Under a 26/21°C environment that time was reduced to around 20 days. Higher temperatures of 32/27°C and 38/33°C did not further reduce the time to anthesis when compared with the 26/21°C environment.

Temperature can also influence the number of flowers produced per inflorescence. Kliewer (1975) found that vines grown at a root temperature of 11°C had significantly more flowers per inflorescence than inflorescences on vines grown at a root temperature of 20°C or higher. Similarly Pouget (1981) reported that decreasing temperature from 25°C to 12°C for pot grown Cabernet sauvignon vines increased the number of flowers per inflorescence by over 100%. Ezzili (1993) and Dunn and Martin (2000) have also observed increases in flower numbers at lower temperatures, although in the case of Dunn and Martin (2000) the relationship between temperature and flower number was weak. In contrast to these studies, Woodham and Alexander (1966) found that warmer soil temperatures increased the number of flowers per inflorescence and also percentage fruitset following anthesis.

Explanations for both the inhibitory (Ezzili 1993, Kliewer 1975, Pouget 1981) and stimulatory (Woodham and Alexander 1966) effects of increased temperature on flower number by these authors at best provide hypotheses for a cause and effect relationship. None of these studies have clearly identified if the temperature effect is 'sensed' by the root or aerial environment of the grapevine. Pouget (1981) speculates that higher temperatures lead to rapid growth of developing shoots which increases the 'speed' of bud burst resulting in less time for flower formation, while lower temperatures reduce the 'speed' of bud burst allowing more time for flower formation. Alternatively May (1987)

hypothesises that higher temperatures increase the production of cytokinin which stimulates the enlargement of early developed flowers, which in turn inhibit the formation of other flowers. Some support for this is provided by Skene and Kerridge (1967) who have demonstrated that changes in root temperature affect the types of cytokinins in xylem sap at bud burst. Another hypothesis proposed here suggests that temperature may control the rate of CHO reserve mobilisation in the roots and trunks of vines and therefore the level of CHO availability to bursting buds at the time of flower formation. In the context of the findings of Ezzili (1993), Kliewer (1975) and Pouget (1981) high temperatures may result in rapid mobilisation of CHO reserves, which hasten bud burst and shoot growth such that there is less time for flower development (Pouget 1981), or that rapid shoot growth consumes much of the available CHO at the expense of flower formation. More simply, the level of CHO reserves available in roots and trunks may influence the number of flowers formed per inflorescence.

2.5.2 Light

Most studies focusing on light effects have used various forms of shading and in general have found that reductions in light intensity reduce the number of inflorescence primordia in latent buds (Baldwin 1964, Dry 2000, Hopping 1977, May and Antcliff 1963, Sommer *et al.* 2000). For example, shading of Sultana (Thompson Seedless) vines for four weeks prior to anthesis has been shown by May and Antcliff (1963) to reduce bud fruitfulness and subsequent yields by as much as 70% in the following season. Similarly, May (1965) has found that heavy shading of the vine canopy consistently reduced bud fruitfulness in terms of both the number and size of inflorescence primordia. In general, controlled environment experiments show that the number and size of inflorescence primordia reduce as light intensity decreases (Buttrose 1969a).

Field observations support the above findings, as it is frequently noted that buds situated inside the canopy of vines are less fruitful than exterior buds which receive more sunlight (May *et al.* 1976). The use of training systems which open up the canopy to light, like the Geneva Double Curtain have been found to improve bud fruitfulness and consequently vine productivity (Dry 2000, Shaulis *et al.* 1966, Sommer *et al.* 2000). Grapevine responses to differing light intensities vary with cultivar. Sultana and Shiraz

require high light intensities while Muscat of Alexandria and Rhine Riesling tolerate lower light intensities (Buttrose 1970). The effects of light intensity on inflorescence primordium formation are independent of temperature (Buttrose 1970).

The mechanism by which light stimulates the initiation of inflorescences remains unclear. Research by Smart *et al.* (1982) indicates the mechanism may be related to light exposure and photosynthesis of the leaf that subtends the latent bud in which inflorescence initiation is occurring. Smart *et al.* (1982) illustrated that increased illuminance of the subtending leaf resulted in increased productivity (inflorescence number and fruit weight) of the shoot that arose from that latent bud in the following season. Such responses imply that light plays just as important role as that of temperature.

The period of natural light exposure (photoperiod) in general does not affect inflorescence initiation in grapevines (Alleweldt 1959, 1964), but there is some evidence that the number of inflorescence primordia per latent bud in vinifera grapes may be greater under long days compared with short days (Buttrose 1969b, Buttrose 1974a). Increasing the period of exposure to high intensity light beyond 12 hours per day was shown by Buttrose (1974a) to increase bud fruitfulness. American grapevine species and hybrids such as *V. labrusca* and Delaware are more sensitive to day length than *V. vinifera* (Kobayashi *et al.* 1966, Sugiura *et al.* 1975). In Delaware (*V. vinifera* x *V. labrusca*) for example, Sugiura *et al.* (1975) found that vines grown in long days formed nearly three times as many inflorescences as those grown in short days. For vinifera grapevines there is no qualitative response to photoperiod, that is, no absolute requirement for flowering. In other words, vinifera grapevines are not dependent on a specific daylength and will eventually initiate inflorescences regardless of daylength, but under long days tend to initiate more inflorescence primordia. Such observations suggest that increased absorption of radiant energy by the leaves of the grapevine, whether it is the result of increased light intensity or the period of light exposure, results in more photosynthesis and therefore the availability of carbohydrate to the initiation of inflorescences within the latent bud.

2.5.3 Water stress

Water stress can reduce the fruitfulness of latent buds (Winkler *et al.*1974). Studies with vines grown in a controlled environment have shown that a high level of water stress reduces the number and size of inflorescence primordia (Buttrose 1974b). There are also reports that water stress may increase the fruitfulness of latent buds (Smart *et al.* 1974). May (1965) suggests that reduced shoot growth and hence foliage density of water stressed vines improves light exposure within the canopy, which in turn improves the fruitfulness of latent buds, particularly those at basal node positions. These responses suggest that water stress may affect fruitfulness indirectly through its influence on leaf photosynthesis and assimilate supply to initiating buds (Loveys and Kriedeman 1973). Specifically, mild water stress reduces leaf to leaf shading (photosynthate production of leaves subtending initiating buds increases), but severe water stress may reduce photosynthate production and supply to initiating buds.

Water stress has also been shown to reduce cytokinin in xylem sap (Livne and Vaadia 1972) and increase abscisic acid levels in leaves and stems (Loveys and Kriedeman 1973). Alteration of hormones under such stress conditions may also be responsible for a reduction in inflorescence initiation and thus fruitfulness. Further discussion on the role of hormones is presented in 2.6.2.

2.5.4 Mineral nutrition

According to Alleweldt (1964) an adequate supply of nitrogen is necessary for inflorescence primordia initiation. Baldwin (1966) has demonstrated that bud fertility of grapevines low or deficient in nitrogen is increased when nitrogen fertiliser is applied. Srinivasan *et al.* (1972) has found that the size of inflorescence primordia is generally unaffected by nitrogen nutrition. However, Muthukishnan and Srinivasan (1974) have found that there can be a significant negative relationship between petiole nitrogen levels and bud fruitfulness. This sort of relationship could be indicative of higher nitrogen stimulating vegetative growth at the expense of reproductive growth. Excessive vegetative growth as a consequence of nitrogen results in dense shaded canopies, which

in turn prevents the exposure of subtending leaves to sunlight with the consequence that inflorescence initiation is inhibited.

Phosphate deficiency is detrimental to inflorescence initiation (Isoda 1964). Kobayashi (1961) has shown that bud fruitfulness can be improved by optimum phosphorus nutrition through its effects on vine vigour. Petiole phosphorus content has also been positively correlated with bud fruitfulness (Muthukishnan and Srinivasan 1974). Studies with radioactive phosphorus have indicated that the vines preferentially accumulate phosphorus in shoot tips and in young buds which subsequently become fruitful (Hiroyasu and Terani 1963, Rao *et al.* 1971). The combination of low nitrogen, high phosphorus and water stress has also been associated with high fruitfulness in Sultana vines (Baldwin 1966).

Srinivasan and Muthukrishnan (1970) suggest that there is also a role for potassium in inflorescence formation in grapevines. Application of potassium to potassium deficient soils has been found to cause a marked increase in the fruitfulness of Concord latent buds (Larsen 1963). Similar responses have been found with Sultana vines in California, where increased bud fruitfulness resulted in significant yield increases, as much as 156% (Christensen 1975). Yield increases like this may also be associated with the positive effects that potassium have on inflorescence primordia size (Srinivasan *et al.* 1972). However, it remains unclear if potassium has direct involvement in inflorescence initiation or that potassium effects are in fact the result of a general increase in grapevine health.

2.6 Physiological factors influencing inflorescence and flower development

2.6.1 Floral induction

Even before initiation of an inflorescence primordium takes place the induction of a precommitted cell or cells in the developing latent bud must occur (Figure 2.3). According to Carr (1967) two different inductive stages controlled by different stimuli could be involved in the flowering of plants. The first is 'primary induction' which is continuously active in plant tissues (depending on the species) and results in vegetative growth. The second is 'secondary induction' which occurs in preselected localised tissues only and results in reproductive growth. Srinivasan and Mullins (1981a) suggest for grapevines that the initiation of anlagen (uncommitted primordia) is controlled by primary induction stimuli, while the conversion of anlagen into inflorescence primordia is controlled by secondary induction stimuli. Both stimuli are probably environmental in nature and mediated by changes in hormones and/or carbohydrates.

Buttrose (1969b) illustrated that induction occurs in latent buds on nodes that are just separating from the shoot tip. Likewise, Botti and Sandoval (1990) state that 'physiological initiation' (induction) begins immediately after the first node separates from the shoot apex. Palma and Jackson (1981) suggest that the chance of each latent bud to become florally induced increases if the temperature is high on the day when the latent bud is on the third node below the apex and in the axil of a leaf 1.5cm in diameter.

Anlagen possess remarkable morphological plasticity, capable of forming into shoot, tendril or inflorescence primordia. In latent buds that became fruitful the following sequence of events occurs. Firstly, the vegetative apex of the latent bud produce anlage (primary induction) which under the same 'induction stimuli' form tendril primordia (2-3 branches), but if the second induction stimulus commences and is of a sufficient magnitude anlagen differentiate into inflorescence primordia (many branches). Thus it follows that the control of inflorescence primordia initiation in grapevines hinges upon the physiology that controls the level of anlagen branching (Srinivasan and Mullins1981a).

2.6.2 Hormones and Carbohydrates

Environmental factors are considered to exert their influence on flowering by evoking changes in the physiology of the plant, particularly the balance of hormones (Steward 1976) and the supply of carbohydrates (Sachs 1977). To prove this requires the establishment of a relationship between the level of endogenous hormones and carbohydrates and the flowering response. In grapes the organs of interest (anlagen, tendril and inflorescence primordia), are very small and difficult to access and conspire to make the extraction of hormones and carbohydrates from latent buds technically very difficult. As a result most work with hormones and flowering in grapevines has been gleaned from the effects of exogenously applied hormones and growth regulators (Srinivasan and Mullins 1981a). In the case of carbohydrates there has been only a small amount of study on their role in the flowering of grapevines. To cover these important areas, brief reviews on hormones and carbohydrates and their influence on grapevine flowering are discussed.

2.6.2.1 Gibberellins

Gibberellins are capable of inducing flower formation in many plant species (Zeevart 1978), but are inhibitory to flower formation in the grapevine (Crozier 1983, Jackson and Sweet 1972). Endogenous gibberellins have been detected in the xylem sap of grapevines (Nakamura and Arima 1969, Niimi and Torikato 1978, Skene 1967) and identified as GA_1, GA_3, GA_5 and GA_9 (Skene 1967). Grapevines are very sensitive to exogenous gibberellins (Weaver *et al.* 1966, Weaver and McCune 1959a). Autoradiography studies have shown that ^{14}C GA_3 applied to grape roots accumulates in leaves and buds (Palma 1985). Applications of GA_3 to field grown vines have been shown by Palma and Jackson (1989) to not only inhibit inflorescence formation, but also reduce the number of flowers produced per inflorescence in the subsequent season. Srinivasan and Mullins (1980a) have found that applications of GA_3 and GA_{4+7} at concentrations as low as 3μmol/L induce the premature bursting of latent buds before winter and stimulate the formation of tendrils from anlagen, but inhibit the formation of inflorescences from anlagen. GA_3 treated grape plants precociously form anlagen at normal node positions and at nodes more proximally situated than is normal. These

anlagen always gave rise to tendrils rather than inflorescences (Srinivasan 1978). Exogenous gibberellin applications can also bring about the transformation of inflorescence primordia into tendrils or tendril-like structures (Mullins 1968). Thus the role of gibberellin in flowering in grapevines is really one of an inhibitor, even though gibberellin stimulate anlagen formation in latent buds.

2.6.2.2 Cytokinins

Srinivasan and Mullins (1978) using *in vitro* culture have shown that isolated tendrils can be made to branch profusely and form inflorescences when treated with the cytokinins Benzyladenine (BA), 6(benzylamino)-9-(2-tetrahydropyranyl)-9H purine (PBA) and Zeatin riboside. With intact vines, repeated applications of PBA (50-200μM) to shoot apices have been found to produce inflorescences in place of tendrils in twelve cultivars of *Vitis vinifera* and six other *Vitis* species. These cytokinin induced inflorescences have been found to produce ripe fruits with viable seeds (Srinivasan and Mullins 1979, Srinivasan and Mullins 1981b). Srinivasan and Mullins (1980a and b) illustrated that the application of a mixture of BA and a growth regulator called Chlormequat could induce normal inflorescences, under low temperature conditions (18-21°C) considered non-inductive to inflorescence initiation. In contrast to the above studies, Palma and Jackson (1989) found no stimulatory effect of the cytokinin 6-benzyl-aminopurine (BAP) on inflorescence initiation in latent buds when applied to grape roots via hydroponic culture. However, they did find that BAP significantly counteracted the negative effects of applied gibberellin on the percentage of buds bearing inflorescences.

The differentiation of the inflorescence primordia and the subsequent formation of grape flowers is influenced by cytokinins (Mullins 1967, 1968, Pool 1975). May (1987) has even suggested that cytokinins may stimulate the enlargement of early developed flowers compared with other flowers. The xylem sap (bleeding) of the grapevine contains high cytokinin activity during bud burst and flowering (Skene and Kerridge 1967) and there is evidence that cytokinin produced in the roots is involved in the regulation of flower differentiation, for example, pistil development (Negi and Olmo 1966). Mullins (1966) has demonstrated with hardwood cuttings, that inflorescence primordia shrivel and die if the emergence of the inflorescence precedes the formation of roots. However, when

roots develop first normal inflorescence development occurs. In rootless cuttings exogenous cytokinins are needed for normal inflorescence development (Mullins 1967). Even fruitset (Weaver *et al.* 1965) and fruit development (Weaver and Van Overbeek 1963) are highly responsive to the application of cytokinins.

Cytokinins are implicated in the control of many aspects of grape reproduction. Therefore it seems that cytokinins have a major regulatory role in the reproductive growth of the grapevine (Srinivasan and Mullins 1981a). However, one has to bear in mind that much of our understanding of cytokinin effects is the result of experimentation where exogenous applications of cytokinins have been used. There is still little data that links endogenous levels of cytokinin to the flowering behaviour of grapevines.

2.6.2.3 Chlormequat

Chlormequat is a plant growth regulator that inhibits the biosynthesis of gibberellin (Lang 1970) and its application to grapevines stimulates inflorescence primordium initiation (Srinivasan and Mullins 1980a). Chlormequat application has also been shown to stimulate tendrils on both primary and lateral shoots of the grapevine to develop into inflorescences (Coombe 1967, Lilov *et al.* 1974, Srinivasan and Mullins 1980a, Sugiura *et al.* 1975). In contrast to inflorescence initiation, anlagen and tendril formation are inhibited by exogenous Chlormequat applications (Srinivasan and Mullins 1980a). Therefore, at an early stage of latent bud development the effect of Chlormequat may be regarded as inhibitory to flowering because of its negative effect on anlagen formation, but at a later stage of development Chlormequat stimulates flowering by negating the inhibitory effects of gibberellin on inflorescence initiation. The effect of Chlormequat on inflorescence formation may also be directly related to cytokinin synthesis, as applications of Chlormequat have been shown to stimulate the biosynthesis of cytokinins in grapevines (Skene 1968, Skene 1970). It is probable therefore that Chlormequat exerts a dual role in the hormonal control of flowering in grapevines through its inhibitory effect on gibberellin biosynthesis and its stimulatory effect on the biosynthesis of cytokinins (Srinivasan and Mullins 1981a).

2.6.2.4 Auxins

Auxins are known to promote cell elongation in many plants, but may under certain conditions influence floral initiation (Zeevart 1978). Jindal and Dabas (1982) have shown that exogenous applications of the auxins, Indole butyric acid (IBA) and 4-chlorophenoxy acetic acid (4-CPA) may increase the fruitfulness of Thompson Seedless latent buds. Palma and Jackson (1989) have illustrated that ^{14}C labelled indole acetic acid (IAA) added to the solution of hydroponically cultured grapevines is absorbed by the roots and translocated to leaves and latent buds. Under hydroponic culture IAA increased the number of latent buds bearing tendrils. In contrast to hydroponically cultured grapevines, Palma and Jackson (1989) found that the injection of the synthetic auxins, naphthalene acetic acid (NAA) and 2,4 dichlorophenoxy acetic acid (2,4-D), into the trunks of field grown grapevines increased the number of inflorescences per latent bud and the number of flowers per inflorescence when compared with non-treated vines. No explanation of how these auxins stimulated flowering in field vines was provided.

2.6.2.5 Carbohydrates

Although hormones have been shown to have a strong regulatory effect on inflorescence and flower formation, there is a growing body of research (although still small compared with that of hormones) that suggest carbohydrates may also have important roles in the flowering of grapevines (Botti and Sandoval 1990, Lavee *et al.* 1967, May 1965, Sommer *et al.* 2000, Srinivasan and Mullins 1976, 1980a, Thomas and Barnard 1937a). May (1965) states that a certain level of carbohydrates produced by the leaves is essential for satisfactory inflorescence formation. Lavee *et al.* (1967) supports this and also suggests that there is a need for carbohydrate accumulation in latent buds for inflorescence initiation. This is also the conclusion of Hopping (1977) after observing reduced fruitfulness of latent buds from Palomino grapevine canopies that had been shaded. Srinivasan and Mullins (1976, 1980a) suggest that the accumulation of starch grains in latent buds may be integral to successful flower formation in grapevines. Similarly, Botti and Sandoval (1990) have found that localised increases in starch levels of latent buds did correlate with inflorescence initiation, in particular starch granules were found in apex cells of latent buds that later contained inflorescence primordia.

Thomas and Barnard (1937a) proposed a positive relationship between the starch concentration of annual wood and fruitfulness at different node positions. This has recently been supported by Sommer *et al.* (2000) who have illustrated that, as starch concentration decreases at higher cane node positions, so does bud fruitfulness. Sommer *et al.* (2000) speculate that inflorescence primordia number and size are positively influenced by carbohydrate supply during the inflorescence initiation process.

Hale and Weaver (1962) have illustrated that the major source of carbohydrates for latent bud development is the subtending leaf. With this in mind work by Vasudevan *et al.* (1998) has shown that shading can reduce leaf, shoot and bud carbohydrates and that reduced carbohydrates may be the cause of a condition known as bud necrosis (BN). Bud necrosis describes a condition where cells in the latent bud die (usually the primary bud). Morrison and Iodi (1990) have observed a lack of starch granules in the latent buds of BN prone vines, but they were unable to determine whether the lack of starch was a causal factor or a consequence of BN. The findings of the studies reviewed above suggest there is a physiological link between starch accumulation in latent buds and inflorescence initiation and the occurrence of BN. Furthermore the findings imply that moderate impairment of starch accumulation in latent buds may first result in a reduction in inflorescence initiation, but when severe impairment occurs, may result in BN.

Having reviewed a possible involvement of carbohydrates in inflorescence initiation, one may also speculate that the supply of carbohydrates from reserves in canes, cordons, trunks and roots before and during bud burst may have a role in determining the number of flowers formed per inflorescence. Scholefield *et al.* (1977) suggests that over-wintering CHO reserves may be directly involved in flower formation after finding that harvest pruning of Sultana vines resulted in inflorescences with fewer flowers in the following season. Scholefield *et al.* (1977) reasoned that the reduction in flowers was a consequence of a reduction in CHO reserves following extensive canopy death after harvest pruning in the previous season. To gain a better understanding of the role of carbohydrates in the flowering of grapevines further research is required. To this end the primary focus of the research presented in this thesis is to investigate the influence of carbohydrate supply on inflorescence initiation and development, flower formation, fruitset and the consequent influence on vine yield.

Chapter 3

General Materials and Methods

3.1 Methods of carbohydrate analysis

3.1.1 Soluble sugar and starch analytical procedures

Two standard colorimetric tests were used to measure the concentration of soluble sugar and starch in grapevine woody tissue. These included the Anthrone test for soluble sugars and *o*-toluidene test for glucose from enzyme digested starch. The extraction and analysis procedures described here are based on Allen *et al.* (1974) and Rose *et al.* (1991) with minor modifications made to the time taken to heat samples and extracts and centrifuge speed. Samples and extracts were generally heated for slightly longer periods of time than those used by Rose *et al.* (1991) to allow for complete extraction and colour reaction. Centrifuge speed was optimised so that complete solid/liquid separation occurred (1048g-force at 2500rpm).

3.1.1.1 Soluble sugar extraction

Freeze dried and finely ground 100mg wood samples were extracted in 10mL of 80% ethanol in a hot water bath (85°C) for 10 minutes (mixing occasionally), centrifuged at 2500rpm for 5 minutes, and the supernatant gently poured off. The pellet was resuspended in 5mL of 80% ethanol and extracted until the supernatant was clear (generally only once more). The supernatants were pooled together and stored in the refrigerator until required. Duplicate 1mL aliquots of soluble sugar extract along with

six glucose and fructose standards in the range of 0 to 1.0mg/mL were mixed with 10mL of Anthrone reagent (1.5g anthrone, 1.0g thiourea and 700mL H_2SO_4 : 300mL H_2O) and heated at 85°C for 15 minutes to allow colour reaction. The absorbency was measured at 625nm using a Helios alpha spectrophotometer (Unicam UV-Vis spectrometry). Standard curves for glucose and fructose were derived using the regression equation:

$Y_g = a + bx$ (Figure 3.1). Where: Y_g = mg glucose or fructose/mL

a = intercept

b = slope

x = absorbance units at 625nm

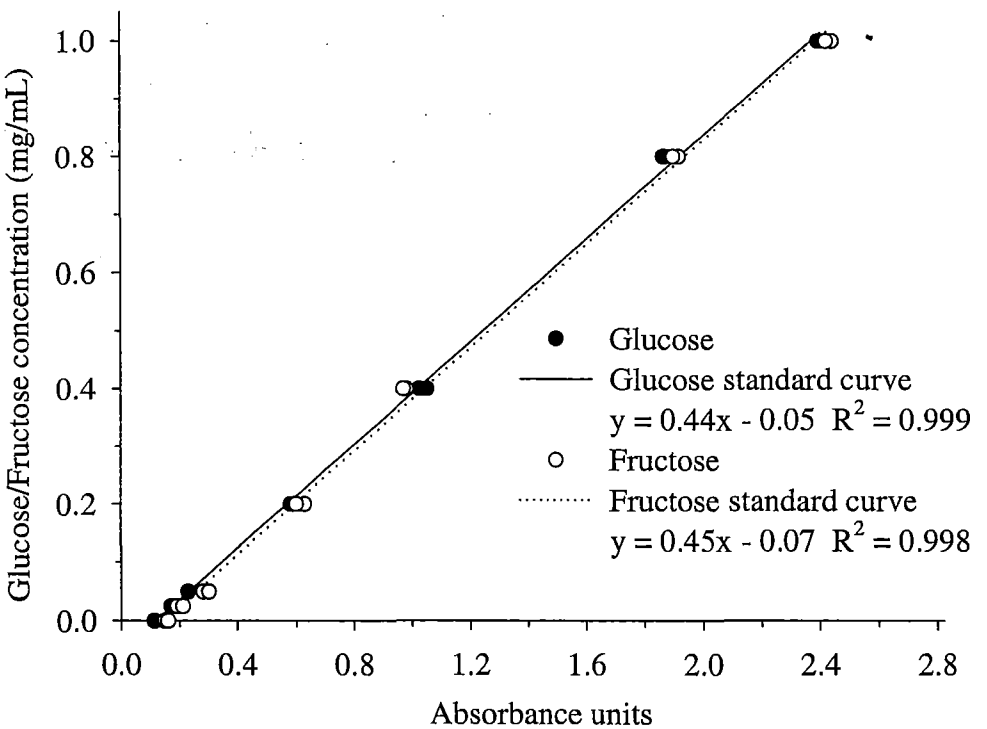

Figure 3.1 Anthrone reagent glucose and fructose standard curves.

Because glucose and fructose formed identical curves with anthrone reagent it was only necessary to run one set of standards (glucose). The soluble sugar (SS) concentrations (% dry weight) were calculated using the following equation (Allen *et al.* 1974).

$$SS(\%\ \text{dry weight}) = \frac{C \times \text{extract volume (mL)}}{10 * \text{aliquot volume (mL)} * \text{sample dry weight(mg)}}$$

Where: C = mg glucose/mL and 10 = percentage conversion factor

3.1.1.2 Starch digestion

Following soluble sugar extraction the remaining solid tissue (pellet) was dried in a hot water bath (85°C for 1-2 hours) to remove ethanol and water. Once dried the samples were resuspended in 5mL of distilled deionised water (ddH_2O) and capped. The samples were heated in a hot water bath (85°C) for 1 hour to allow for starch gelatinisation, then quickly cooled in a cold water bath (10°C). One mL of starch digestion solution (400 enzyme units/mL α-amylase (Sigma A-2643), 2 enzyme units/mL amyloglucodase (Sigma A-3514), adjusted to pH 5.1 using sodium acetate buffer) was added to the samples and incubated at 50°C for 48 hours, mixing occasionally. After incubation the samples were centrifuged at 2500rpm for 5 minutes and the supernatant collected for colorimetric analysis. Supernatant aliquots of 0.1mL (in duplicate) along with six glucose standards in the range of 0 to 3.4 mg/mL were mixed with 5mL of *o*-toluidene reagent (1.0g thiourea, 940mL glacial acetic acid and 60mL *o*-toluidene) capped and heated for 20 minutes at 85°C to allow for colour reaction. The absorbency was measured at 635nm using a Helios alpha spectrophotometer (Unicam UV-Vis spectrometry).

The standard curve from the glucose standards was derived using the regression equation $Y_g = a + bx$. The glucose concentrations in the sample were calculated by substituting the absorbance readings into the x variable in the regression equation (Figure 3.2). The weight (mg) of starch in the sample was calculated using the equation:

mg of starch/mg of sample = $Y_g d_f v h_f / dw$ (Rose *et al.* 1991).

Where: Y_g = glucose concentration (mg/mL)

d_f = dilution factor (if necessary, e.g. 10 for 1 : 9)

v = original volume of starch extract
(5mL ddH_2O + 1mL starch digestion solution)

h_f = starch hydrolysis factor (0.9) (Volenec 1986)

dw = original sample dry weight (mg)

The weight (mg) of starch in the sample was converted to a % dry weight basis using the following equation:

$$\% \text{ dry weight} = \frac{\text{mg starch/sample}}{\text{sample weight (mg)}} * 100$$

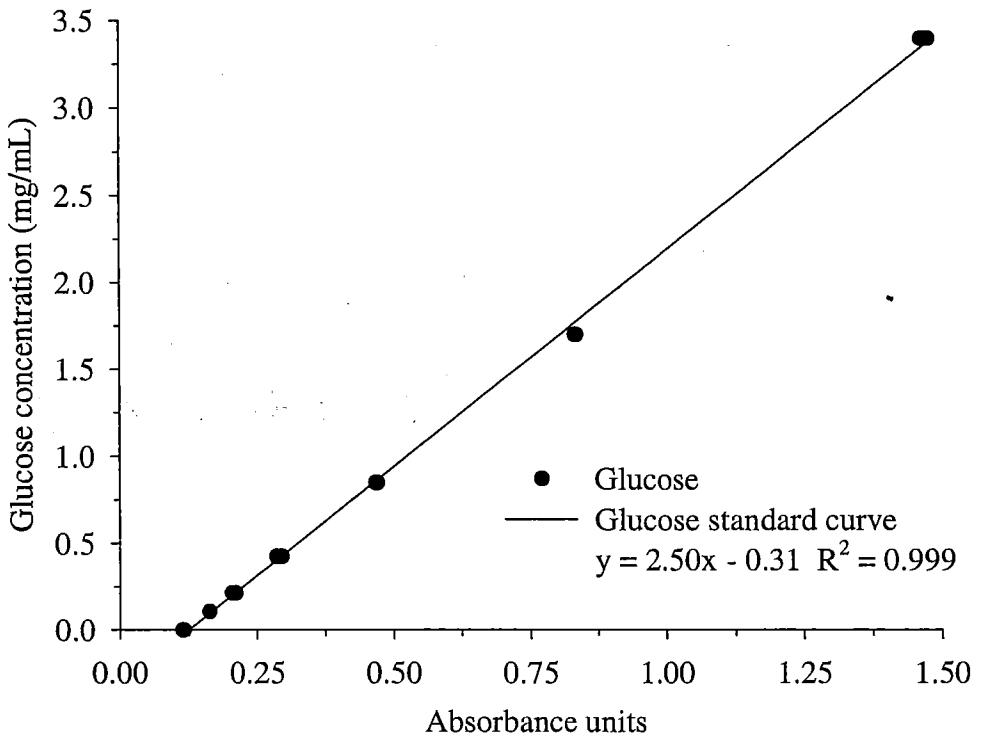

Figure 3.2 *o*-toluidine reagent glucose standard curve.

Preliminary analyses were performed to test the efficacy of the starch enzyme digestion process. This involved 'spiking' a standard bulk wood sample with high grade starch. The starch concentration of the standard bulk wood sample was raised from a natural concentration of 6.9%Dwt to 10, 15, 20 and 25%Dwt by calculating the amount of starch required to be added to 100mg standard wood samples. The added starch was thoroughly mixed into the sample and then frozen for a week before being analysed. The standard and spiked samples were then analysed using the procedures described in 3.1.1.1 and 3.1.1.2. Results from the analysed standard and spiked samples returned starch concentrations of 6.8, 9.8, 15.6, 19.4, 24.4%Dwt respectively. These results illustrated that the starch enzyme digestion process was able to accurately and reliably detect changes in the starch concentration of wood samples.

3.2 Assessing carbohydrates in different parts of the grapevine

As has been reviewed in 2.3 research literature from various viticultural regions around the world has illustrated that a wide range of environmental and management factors during the growing season influence carbohydrate reserves in above ground portions of the vine, for example, seasonal conditions (Bains *et al.* 1981, Winkler and Williams 1945), crop load (Balasubrahmanyam *et al.* 1978, Weaver and McCune 1960), leaf area/defoliation (Candolfi-Vasconcelos and Koblet 1990, Koblet *et al.* 1993), vine nutrition (Korkas *et al.* 1996a and b), pests and diseases (Rühl and Clingeleffer 1993, (Ryan *et al.* 2000), irrigation (Rühl and Alleweldt 1990) and vine pruning/training (Koblet *et al.* 1993, Schultz *et al.* 2000, Williams 1996). However, most of this literature has only reported on carbohydrates in canes, cordons and trunks. Less information is available on the concentrations of carbohydrates (starch and sugar) in grapevine roots, despite their importance as a primary CHO reserve organ. There is also little information in the literature that assesses the variation in starch and sugar concentrations within, and between, different vine parts and the contribution of each part to the total carbohydrate reserve pool of the vine.

To gain a better understanding of the variation in carbohydrates within, and between, different vine parts and their contribution to the total carbohydrate reserve pool of the grapevine pre-experimental investigations were carried out on grapevines at the Lincoln University research and teaching vineyard (latitude 43° 39' south, longitude 172° 28' east). The results of the investigations were used in the development of an unbiased wood sampling protocol for experimental vines.

3.2.1 Vine material

Six established (> 5 years old) cordon spur pruned Chardonnay vines (own roots) were completely removed from the field in midwinter (July 1998) and separated into shoots, cordon, trunks and roots and their fresh weights were measured. Complete root recovery was attempted by digging down deep into the soil profile. Small samples of fresh wood from the various vine parts were taken for carbohydrate analysis and included:

- Six trunk samples from top, middle and base of both north (exposed) and south (shaded) sides

- Three cane samples from internodes 2, 6 and 10 (typical cane region)

- Three cordon samples from end, middle and base

- Three root samples from the following diameter categories:
  3 to 5mm, 10 to15mm, ≥ 20mm (representing young to old roots respectively)

The fresh wood samples were freeze dried for 48 hours, then ground to a powder using a ring grinder (Rock labs) and stored at -20°C until analysed. The concentrations of starch and sugar were determined using the methods described in 3.1. The remaining fresh vine parts were dried in an oven (70°C for 48 hours) and then weighed. In the case of the roots they were first separated into the three size categories before drying. Calculations of CHO content for each vine part were based on CHO concentration data and dry weights. All vine weights and carbohydrate data were analysed using general ANOVA from the Genstat statistical package (Genstat 5 Release 4.1. Copyright 1997, Lawes Agricultural Trust, Rothamsted Experimental Station). Mean separations were determined utilising least significant difference (lsd) at the 5% level of significance.

3.2.2 Results

Trunk carbohydrate analyses revealed that soluble sugar concentration at the base of the trunk was significantly lower than the top or middle of the trunk (Table 3.1). There were no differences in starch concentration between the three positions, but as a result of differences in soluble sugar, total CHO concentrations were lower at the base of the trunk. There was no significant difference in any CHO form between north (exposed) and south (shaded) sides of the trunk (Table 3.1) nor was there any interaction between trunk position and side.

Table 3.1 The main effects of trunk position and trunk side on the concentration of soluble sugar, starch and total CHO in Chardonnay grapevines during midwinter.

Trunk Position	Top	Middle	Base
Soluble sugar (%Dwt)	13.5 a[1]	13.1 a	10.6 b
Starch (%Dwt)	11.1 a	11.3 a	11.7 a
Total CHO (%Dwt)	24.6 a	24.4 a	22.3 b
Trunk Side	North side		South side
Soluble sugar (%Dwt)	12.1 a		12.8 a
Starch (%Dwt)	11.3 a		11.4 a
Total CHO (%Dwt)	23.2 a		24.2 a

[1]Means within the same row with the same letter are not significantly different at $P \leq 0.05$.

Shoot soluble sugars were significantly higher at internode two than either internode six or ten, but there were no differences in starch concentration between the three internode positions (Table 3.2). As a consequence of higher soluble sugars at internode two, total CHO at internode two was higher than at internode six or ten.

Analysis of cordon samples showed there were no significant differences in soluble sugar, starch or total CHO concentration between the end, middle or base positions of the cordon (Table 3.2).

Results from root samples revealed there were no significant differences in soluble sugar concentration between the three root size categories (Table 3.2). Starch and total CHO however, increased in concentration as the root size became larger, indicating that the largest roots (≥ 20mm) of the grapevine had the highest concentration of reserve carbohydrates (Table 3.2).

Table 3.2 The effects of shoot internode and cordon position and root size on the concentration of soluble sugar, starch and total CHO in Chardonnay grapevines during midwinter.

Shoot internode position	**Two**	**Six**	**Ten**
Soluble sugar (%Dwt)	16.1 a[1]	14.7 b	14.6 b
Starch (%Dwt)	8.1 a	8.2 a	7.4 a
Total CHO (%Dwt)	24.2 a	22.9 b	22.0 b
Cordon position	**Base**	**Middle**	**End**
Soluble sugar (%Dwt)	13.8 a	13.1 a	13.0 a
Starch (%Dwt)	9.8 a	11.3 a	10.3 a
Total CHO (%Dwt)	23.6 a	24.4 a	23.3 a
Root diameter category	**3-5mm**	**10-15mm**	**≥ 20mm**
Soluble sugar (%Dwt)	6.2 a	5.8 a	6.1 a
Starch (%Dwt)	8.8 a	17.4 b	19.6 c
Total CHO (%Dwt)	15.0 a	23.1 b	25.7 c

[1]Means within the same row with the same letter are not significantly different at $P \leq 0.05$.

The trunk was the largest part of the vine accounting for 43% of the vine's total dry weight biomass and consequently contained more starch, soluble sugar and hence total CHO than any other part of the vine (45% of total vine) (Table 3.3). The cordon was the next largest part of the vine, accounting for 24% of the vine's total dry weight biomass. The roots accounted for 19% of the vine's total dry weight biomass, but contained more starch (28% of total vine) than the cordon (21% of total vine). Shoot growth accounted for 15% of the vine's total dry weight biomass and contained significantly lower amounts of starch than any other part of the vine (Table 3.3). The cordon had the highest dry weight proportion of all vine parts at 58%, while roots had the lowest dry weight proportion at just 42% (Table 3.3).

Table 3.3 The fresh and dry weights of Chardonnay grapevine shoots cordon, roots and trunk and their soluble sugar, starch and total CHO content during midwinter.

Vine part	Shoots	Cordon	Roots	Trunk
Fresh weight (g)	508.0 a[1]	712.0 b	812.0 b	1384.0 c
Dry weight (g)	260.4 a	412.8 b	341.5 ab	757.6 c
% Dry weight	51 a	58 b	42 c	55 d
% of total vine	15 a	24 b	19 a	43 c
Soluble sugar content (g)	39.5 a	54.5 b	20.8 c	94.2 d
Starch content (g)	20.8 a	43.0 b	45.0 b	87.2 c
Total CHO content (g)	60.3 a	97.5 b	65.8 a	181.4 c
% Sugar content of total vine	19 a	27 b	12 c	46 d
% Starch content of total vine	10 a	21 b	28 c	44 d
% Total CHO content of total vine	15 a	24 b	20 ab	45 c

[1]Means within the same row with the same letter are not significantly different at $P \leq 0.05$.

3.2.3 Discussion and sampling protocol development

Large variations in CHO concentrations were found between vine parts, for example in trunks starch concentration averaged 11.5% while in shoots it was 8%. Large variation was also evident within vine parts, for example in small roots 3-5mm in diameter starch concentrations were 8.8%, while in large roots ≥ 20mm in diameter starch concentrations were as high as 19.6% (Table 3.2). Overall midwinter trunk soluble sugar, starch and total CHO concentrations averaged 12.5%, 11.4% and 23.8% respectively (Table 3.1). These concentrations are in part comparable to those reported by Winkler and Williams (1945) (soluble sugar 8.4%, starch 14.8% and total CHO 23.3%) from the trunks of *Vitis vinifera* Carignane. Koblet *et al.* (1993) found with *Vitis vinifera* Pinot noir that midwinter trunk soluble sugars, starch and total CHO averaged 14.5%, 2.5% and 17.0% respectively, while Williams (1996) showed for Thompson Seedless that concentrations were 4.2%, 14% and 18.2% respectively. The data of Koblet *et al.* (1993) and Williams

(1996) are quite different from those shown in Table 3.1 and suggest that comparisons between the trials cannot be made because of differences in grape variety, viticultural management and climate.

Analysis of shoot wood shows that the concentrations of soluble sugar and total CHO at the base of the shoot (internode two) were higher than those at higher node positions (internodes six and ten). Similar patterns have also been shown by Winkler and Williams (1945) and Sommer *et al.* (2000), for example at the base of *Vitis vinifera* Carignane shoots, soluble sugar, starch and total CHO were 11.4%, 12.2% and 23.6% respectively, while at a higher node position they were 10.2%, 11.3% and 21.5% respectively (Winkler and Williams 1945). Koblet *et al.* (1993) and Korkas *et al.* (1996b) have reported much lower shoot CHO concentrations than those presented in Table 3.2. Koblet *et al.* (1993) found that the concentrations of soluble sugar, starch and total CHO at the fifth internode of Pinot noir shoots was 10%, 3.1% and 13.1% respectively, while, Korkas *et al.* (1996b) reported concentrations of 9.7%, 4.9% and 14.6% respectively in the shoots of White Riesling. The differences highlighted here are likely to be a reflection of both grape variety and viticultural management. Both Koblet *et al.* (1993) and Korkas *et al.* (1996b) investigations were carried out on vines growing in Central Europe (short growing season cool climate viticulture).

The concentration of starch and total CHO was greater in larger roots (Table 3.2). Similar observations have been reported by Winkler and Williams (1945), for example roots 5.6mm in diameter had a starch concentration of 14%, while roots 16.0mm in diameter had a starch concentration of 16.5%. Williams (1996) has shown for Thompson Seedless that winter root soluble sugar, starch and total CHO concentrations were 5.5%, 18% and 23.5% respectively. These concentrations are very similar to those obtained for the larger sized roots in this study of Chardonnay vines (Table 3.2).

In developing an unbiased protocol for the sampling of vine wood for carbohydrate analysis, the primary concern is that samples are representative of the part of the vine under examination. In terms of perennial woody parts of the vine, the results from this study suggest trunk, cordon and shoot samples should be taken from a middle position. In the case of the trunks it is important to avoid the base which may have lower carbohydrate concentrations. With shoots, a middle position (internode six) should be

sampled, as sampling from lower internode positions may result in higher carbohydrate concentrations that are not representative of the majority of the shoot. Roots are systematically variable and when sampling a decision must be made to standardise root size first. It is recommended that roots from 10 to 15mm in diameter are sampled. This size represents a root of several years of age, but one that is not a main root and therefore may be sampled once with minimal, or no effect on the mature vine.

In contrast to the dry weight results presented in Table 3.3, Mullins *et al.* (1992) found for 10 year old Chenin blanc vines trained to a similar cordon system that the cordon was the single largest part of the vine, accounting for 29% of the vine's total dry biomass. Both trunk and roots accounted for 25% of the dry biomass each, while new shoots accounted for 21%. Comparing trunk and root data of Mullins *et al.* (1992) with that presented in Table 3.3 revealed that there has almost been a complete reversal between trunks and roots in terms of their contribution to the total carbohydrate reserve pool of the vine. In the Chenin blanc vines the roots were the prominent store of reserves (49%), whereas in this study, the trunk was the most significant store of reserves (45%). Mullins *et al.* (1992) data showed that an average 10 year old Chenin blanc vine had a dry biomass of 12kg while the dry biomass of the younger Chardonnay vines of this study was only 1.8kg. Reasons for these differences are most likely related to vine age, vigour and grape variety. Although comparisons are limited because of variety difference and vine age, the higher proportion of carbohydrate reserves partitioned to the roots of mature Chenin blanc vines highlights the importance of the root system as a store of CHO reserves.

In concluding this investigation the results have illustrated that particular parts of the grapevine have a more specialised role in storing carbohydrate reserves than others, for example larger roots have higher starch and total CHO concentrations than any other part. However, the biomass of a particular vine part also has an influence on reserve CHO pools, for example the trunk, although not having the highest concentrations of carbohydrates contained the largest amount of reserve CHO. Variation in CHO concentrations within vine parts has highlighted that some parts of shoots and trunks should be avoided if a representative sample is to be attained, while standardisation of root size before sampling must be considered, given the systematic variation in CHO concentrations across root sizes.

3.3 Estimating flower number per inflorescence

Counting the number of individual flowers per inflorescence is by no means an easy task considering that flower buds pre-bloom are only a few millimetres wide and may number a thousand or more per inflorescence. Often damage to flowers and inflorescences may occur in the process of counting thus ruining attempts to measure flower number and follow post-bloom events on the same inflorescence/cluster. Therefore a reliable and reasonably non-intrusive technique that can accurately estimate total flower number per inflorescence must be devised.

3.3.1 Techniques for estimating flower number

A search of the literature reveals that only two techniques have been successfully used in research trials over the last couple of decades. The conventional approach to estimating flower number per inflorescence (still attached to the vine) has been to seal the inflorescence within net bags allowing for all the flower caps (calyptra) to be collected as they detach and fall free of the flower. Candolfi-Vasconcelos and Koblet (1990) successfully used gauze bags to determine flower/fruitlet drop over time and fruitset. Although this technique can work it has a high labour input in terms of gently enclosing inflorescences without damage as well as counting all the flower caps and fruitlets collected.

Alternatively May (1987) has estimated flower number per inflorescence by determining the relationship between the number of flowers counted on branches of the inflorescence and the total flower number per inflorescence. May (1987) sampled almost one hundred inflorescences and counted the number of flowers on branches one to seven and then regressed the sum of pairs of branches (eg. branch one + branch two) against total flower number. The resulting relationships were linear with coefficients of determination (R^2) ranging from 0.81 to 0.91. Branch pair three + four accounted for over 90% of the variation in total flower number, while the branch pair one + two accounted for approximately 80% of the variation in total flower number. May (1987) concluded that this technique may be sufficiently robust to be used in forecasting yield.

To test May's technique under New Zealand's cool climate environment a population of small to large sized inflorescences were removed from Chardonnay grapevines approximately 1-2 weeks before bloom in the 1997/1998 season. The number of flowers on the first, second and third branches of the inflorescence were counted (Figure 3.3) and then regressed against total flower number per inflorescence. The selection of the first three branches of the inflorescence was done to help simplify the estimation method.

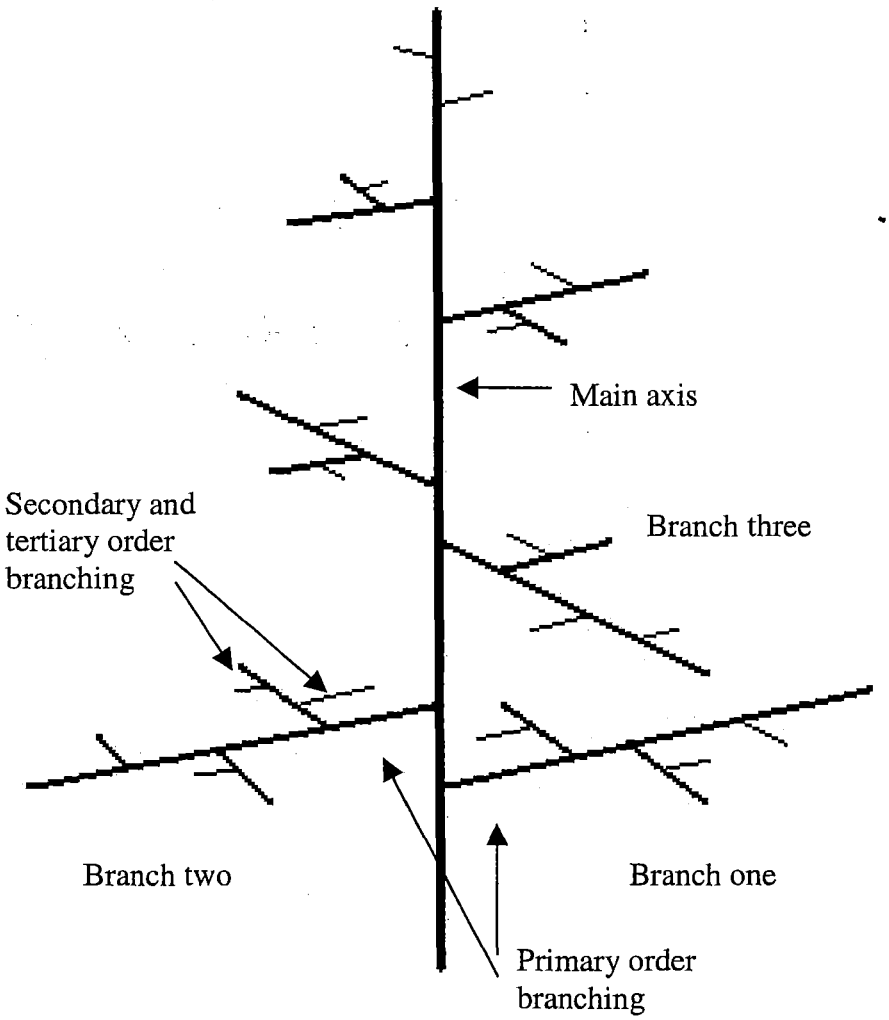

Figure 3.3 Chardonnay inflorescence branching.

3.3.2 Results and Discussion

The results of the regression analysis revealed that the number of flowers on branches one, two, three and one + two all had strong linear relationships with total flower number per inflorescence with R^2 values ranging from 0.87 to 0.93 (Figure 3.4). It was expected

that the branch one + two relationship would have the highest coefficient of determination (R^2) because branch one + two represented a higher proportion of the inflorescence than any branch on its own. The coefficients of determination are remarkably similar to those of May (1987), which suggests the linear relationship is tightly fixed for inflorescences regardless of season or location.

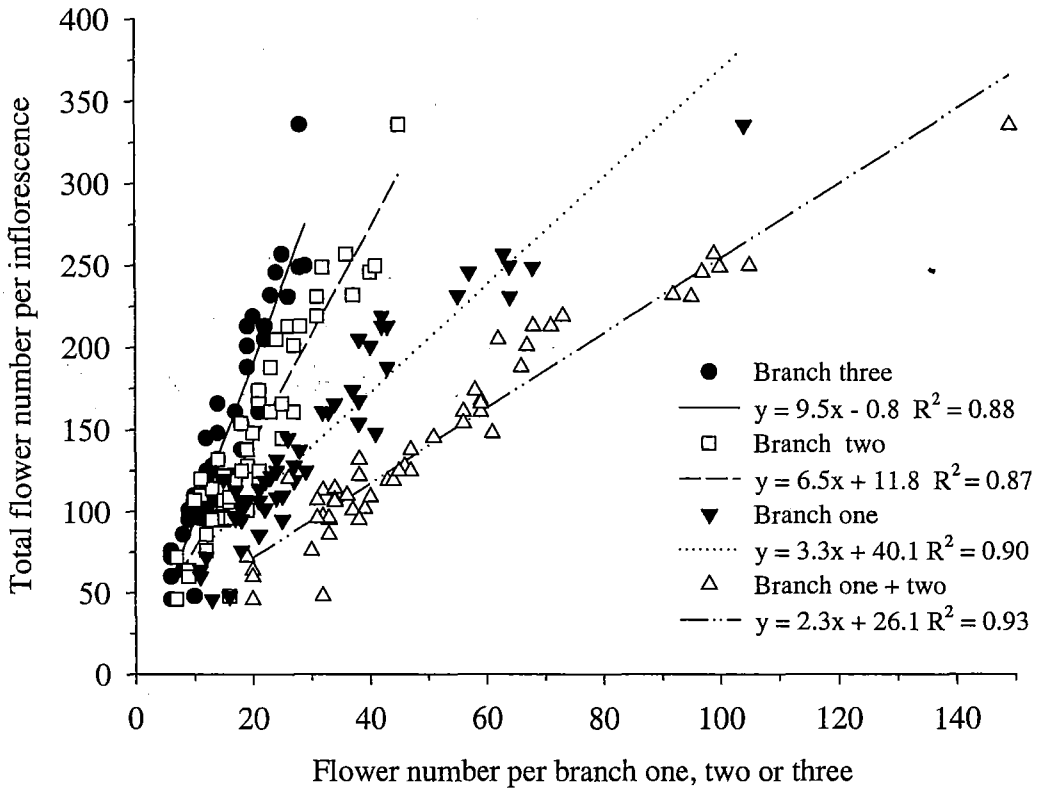

Figure 3.4 The relationship between branch one, two, three and one + two flower number and total inflorescence flower number (1997/1998).

Because of the arrangement of the inflorescence (Figure 3.3) it became progressively more difficult to count flowers on higher branches (branch two and three). Higher branches are closer together and therefore it becomes physically more difficult to separate out and count flowers without inadvertently breaking individual flowers off. Because of this reason and the fact that branch one flower number generated a stronger relationship, a decision was made to only use the branch one relationship (Figure 3.5) for estimation of total flower number per inflorescence in experimental vines.

Although the coefficient of determination (R^2) was high for the branch one relationship (Figure 3.5), 95% prediction intervals indicated that there was variation (error) associated

with the prediction of total inflorescence flower number (Figure 3.5). The associated variation or error meant there was a degree of limitation on the use of this relationship. In other words, the relationship can be used to predict flower number as long as the experimenter is prepared to tolerate the degree of associated error with the prediction. The degree of error can be reduced by increasing sample size, in this example (Figure 3.5) a minimum of fifty inflorescences were counted. In order for the relationship to be reasonably representative of all inflorescences on grapevines a sufficiently large sample size must used. The relationship shown in Figure 3.5 was used to estimate total flower number per inflorescence for the experiment described in Chapter 4.

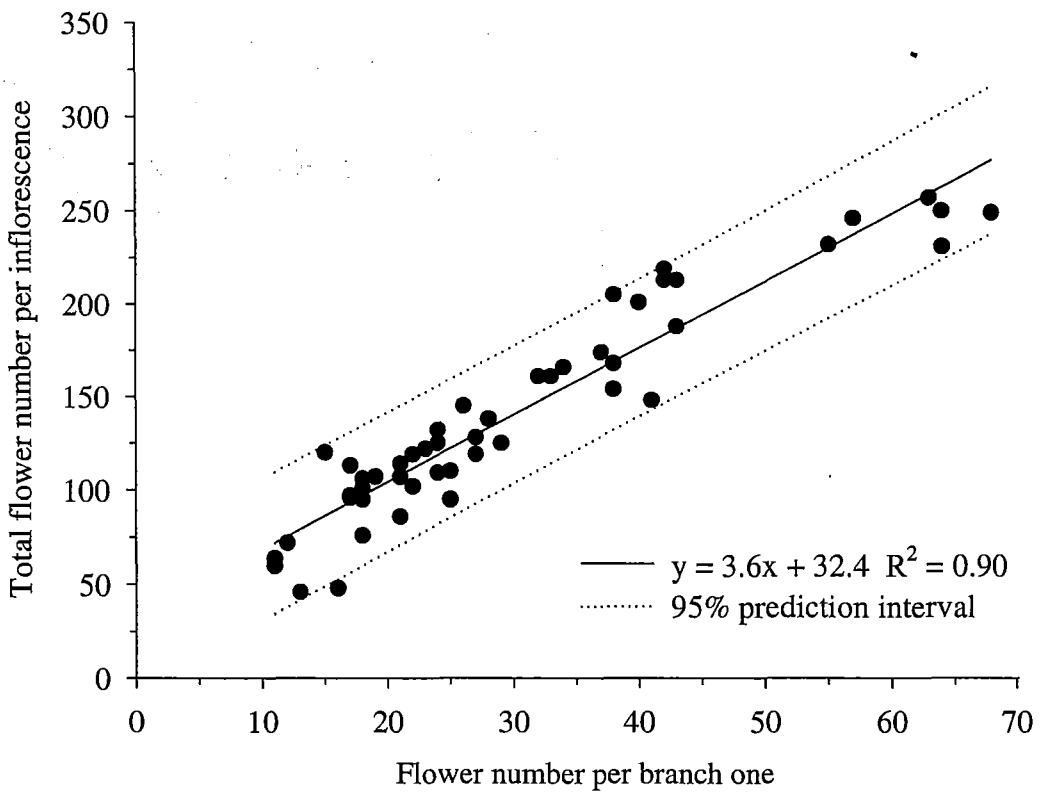

Figure 3.5 The relationship between branch one and total inflorescence flower number with 95% prediction interval (1997/1998).

Even though the relationship between branch one and total inflorescence flower number was strong an assessment of the relationship with the actual number of flowers formed on these inflorescences needed to be determined to illustrate that the prediction method was (within excepted levels of error) accurately estimating total inflorescence flower number. A new predictor relationship was determined in the 1998/1999 season using a population of fifty Mendoza Chardonnay inflorescences (small to large in size) from non-

experimental vines. Results from the new predictor relationship (Figure 3.6) revealed a strong relationship between branch one and total inflorescence flower number with a high R^2. The slope was much higher than that of the previous season's relationship (Figure 3.5). The difference in slopes is either a reflection of different seasons or clone of Chardonnay.

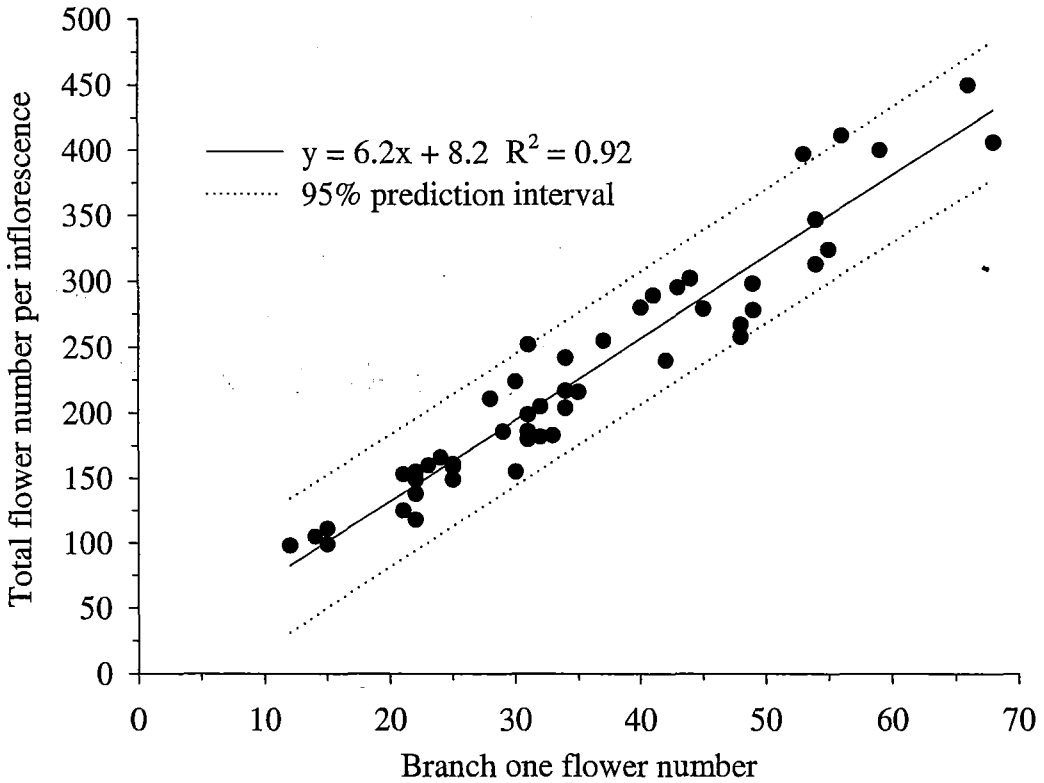

Figure 3.6 The linear relationship between the flower number on branch one and total flower number per inflorescence(1998/1999).

Following the generation of the new predictor relationship, the number of flowers on branch one of the inflorescences of experimental vines (see Chapter 5) were counted and an estimation of total inflorescence flower number was calculated. Immediately after this the same inflorescences were sealed in fine net bags so that all the flower caps could be collected as they fell off the flowers (Plate 3.1). Five weeks after bloom the net bags were gently removed from the inflorescences and the number of flower caps and fruitlets counted (Plate 3.2). Flower caps per inflorescence were then regressed against estimated flower number per inflorescence. The slope of the resulting linear regression (Figure 3.7) was close to one (0.9) suggesting that estimated flower number was very similar to actual flower number, however with an intercept of 43, estimated flower number was higher

than the number of caps counted for the same inflorescence (Figure 3.7). This led to the conclusion that May's (1987) method of flower estimation was over-estimating the actual number of flowers formed per inflorescence. This assumed that the number of flower caps collected in the net bags was an exact reflection of the number of flowers formed per inflorescence. However, observations of some clusters at harvest revealed that there were still some flower caps trapped in the clusters, consequently the number of caps collected at 5 weeks post bloom was in fact under-estimating the number of flowers formed per inflorescence. Because of this, and the fact that the estimation relationship had been successfully used before, it was decided that May's (1987) method would be used in preference to the net bag method. Consequently flower numbers shown in Chapter 5 were estimated using the predictor relationship shown in Figure 3.6. May's (1987) flower estimation method had several advantages over the net bag method; firstly labour input was significantly reduced - less counting and secondly, more samples could be measured to reduce the experimental sample error component.

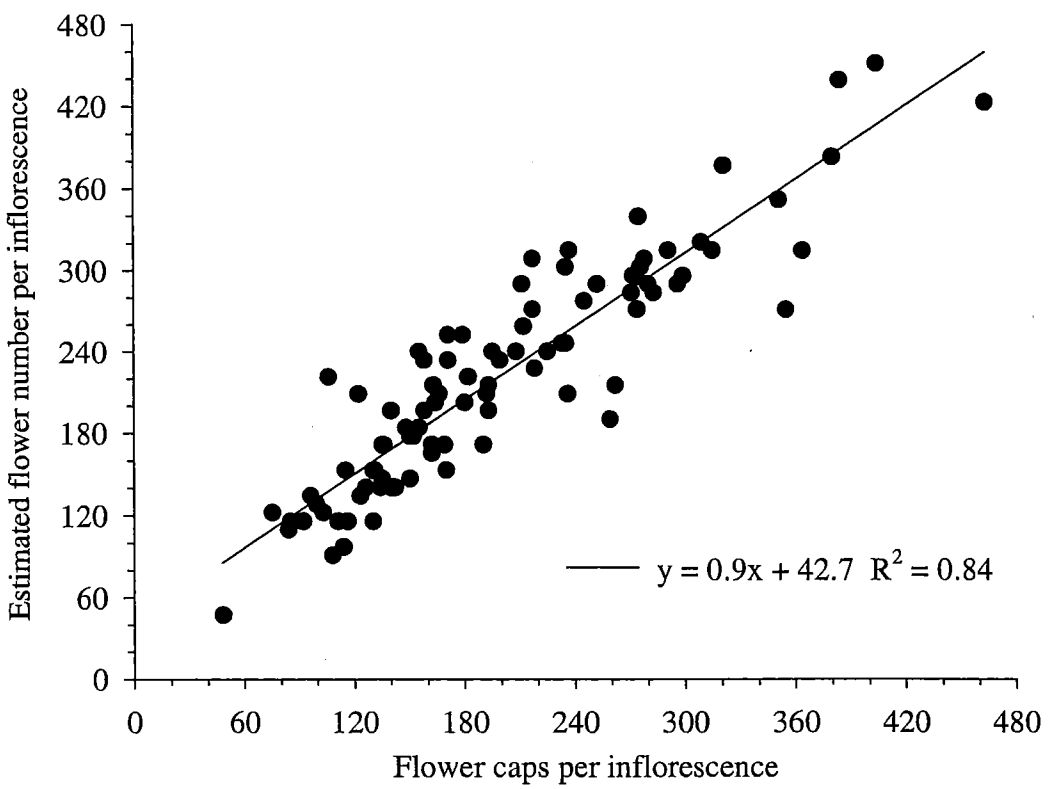

Figure 3.7 The linear relationship between flower caps and estimated flower number per inflorescence (Mendoza Chardonnay - 1998/1999)

Plate 3.1 Inflorescences sealed in net bags at the start of bloom.

Plate 3.2 Flower caps (left) and aborted fruitlets (right) collected from a single inflorescence five weeks after bloom.

Chapter 4

The influence of the intensity of vine defoliation on return bloom and yield

4.1 Introduction

Leaf removal or partial defoliation is a term in viticulture used to describe the practice of removing leaves from around fruit clusters and/or throughout the vine canopy during the growing season. The practice is now extensively used in cool climate viticultural regions all over the world as it has several major advantages for improving fruit quality (Smart and Robinson 1991). Firstly, it increases spray penetration into the fruit zone to prevent diseases such as Botrytis bunch rot and powdery mildew, as well as increasing wind and light exposure to hasten fruit drying after rain to reduce the risk of Botrytis infection (Bettiga *et al.* 1989, English *et al.* 1989, 1990 and Gubler *et al.* 1987). Secondly, leaf removal improves cluster sunlight exposure which in turn improves berry sugar and acid composition (Bledsoe *et al.* 1988, Candolfi-Vasconcelos and Koblet 1990, Crippen and Morrison 1986, English *et al.* 1989, Kliewer and Lider 1968, Koblet 1987, Smart 1987), as well as berry colour (anthocyanins) (Dokoozlian and Kliewer 1996, Phelps 1999).

The potential crop from an individual vine is made up of several yield components (see Figure 4.1), these include both reproductive and vegetative parts of the grapevine (May 1987). The first component fixed is the number of inflorescence primordia initiated per latent bud in the current season. At the start of the following season the number of buds that burst and grow into shoots that carry the inflorescences will determine the number of inflorescences per vine. While buds are bursting, the number of flowers formed on each

inflorescence is determined (May 2000 and Pratt 1971). The proportion of flowers that turn into berries during the fruitset process will then determine the number of berries per cluster (formerly referred to as the inflorescence). Following fruitset, the potential yield is influenced by berry size (weight). The weight of the berry multiplied by berry number will determine cluster weight. Finally the cluster weight multiplied by cluster number per vine will determine individual vine yield (Figure 4.1).

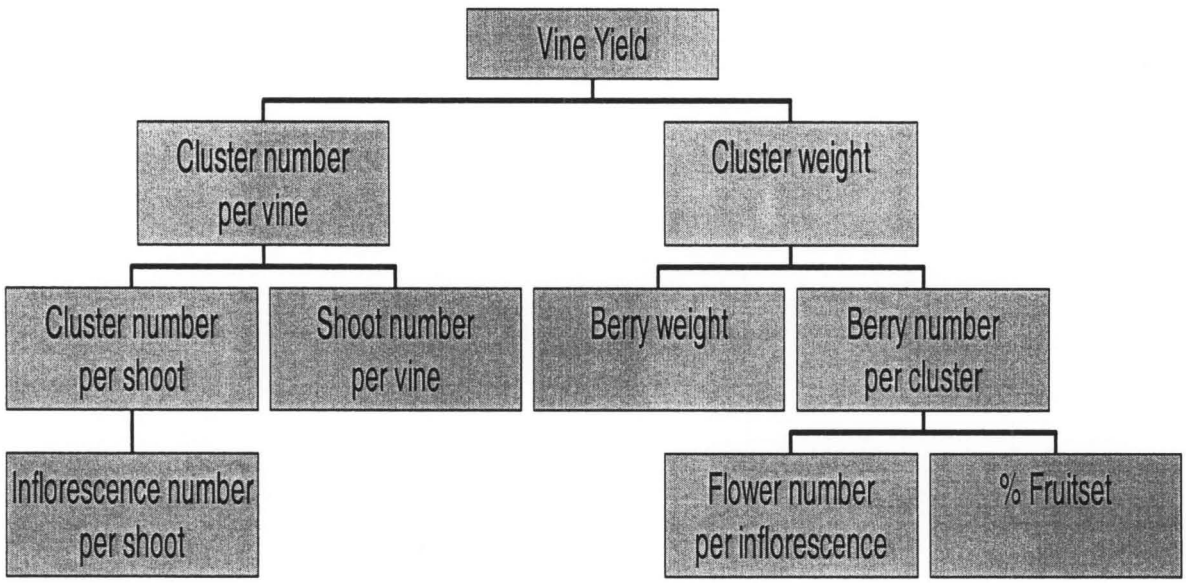

Figure 4.1 The primary components of grapevine yield.

Although we have a good appreciation of the benefits of leaf removal/defoliation on fruit quality in the current season, and also a clear understanding of what constitutes grapevine yield, we do not know the impact of leaf removal/defoliation on return bloom, in terms of individual yield components, or vine growth or cropping in the following season under New Zealand's cool climate environment. It is conceivable, that many of the yield components outlined in Figure 4.1 may be negatively affected by the timing and/or intensity of leaf removal/defoliation in the season previous to fruiting, with the consequence that vine yields are significantly reduced. Therefore the aim of this experiment was to assess the effects of the intensity of vine defoliation on individual grapevine yield components and yield in the following season under the cool climate viticultural conditions of New Zealand's South Island.

4.2 Materials and Methods

4.2.1 Defoliation

Ten days after bloom during the 1996/1997 growing season mature Chardonnay vines (clone unknown, own roots, VSP trained approx. 11 nodes per cane) growing in the Lincoln University vineyard were defoliated by removing either 0% (no defol.), 33%, 66% or 100% of the leaves above the fourth node on every shoot per vine. These percentages of defoliation equated to removing none, every third, every two out of three, or all the leaves per shoot respectively above the fourth node. The four basal leaves were retained to provide some leaf area to keep the vine alive and protect current crop from sunburn. Each treatment (one vine) was replicated eight times in a randomised block design, thus 32 vines were used in the experiment. All the shoot tips were left to grow but newly developing leaves were regularly removed according to treatment. Other than this the vines were subject to standard vineyard care – spraying for disease control and irrigation under dry conditions. Data on clusters per vine and yield during the defoliation were not available. At the end of the season (June 1997) the vines were pruned back to a standard VSP system, two canes with ten nodes on each and two head spurs.

4.2.2 Inflorescence, flower and cluster measurements

To get an early indication of the effects of defoliation on inflorescence number per node (bud fertility), one shoot from the prunings of each vine that best represented a typical cane was sectioned into single node cuttings straight after pruning (June 1997) (May and Antcliff 1973). The single node cuttings were placed in trays of water in a heated glasshouse and left to grow into a shoot (Plate 4.1). Once sufficient growth of the shoots had occurred the number of inflorescences at each node was counted.

At one and half months after bud burst in the following season (November 1997) a count of inflorescence numbers on all the developing shoots for each treatment vine was made, this was then compared with the earlier single node cutting data. Just prior to bloom (December 1997) three randomly selected inflorescences per vine were then labelled and

a count of branch one flower number was made. Total flower number for the selected inflorescences was then estimated using the branch one prediction method (see Chapter 3.3, Figure 3.5). Just prior to main harvest (April 1998) the labelled clusters were harvested and weighed. The berry number on each cluster was counted and the remaining rachis weighed. In order to calculate mean berry weight the weight of the rachis was subtracted from cluster weight, the resulting total berry weight was then divided by berry number for the same cluster. At the end of the season once the vines had become dormant (June 1998) they were pruned to standard VSP system, two canes with ten nodes on each and two head spurs. Calculation of vine capacity (estimate of annual dry matter production) was performed using the following formula: Vine capacity = 0.55(pruning weight) + 0.25(fruit weight), where 0.55 = percentage dry matter content of prunings and 0.25 = percentage dry matter content of fruit (Winkler *et al.* 1974). Yield to pruning weight ratios (Ravaz index, Ravaz 1930) were also calculated for each treatment vine.

Plate 4.1 Single node cuttings busting bud in the heated glasshouse.

4.2.3 Statistical analysis

All flower, fruit and yield data from the experiment were analysed using general ANOVA testing for polynomial (linear) significance using a Genstat statistical package (Genstat 5 Release 4.1. Copyright 1997, Lawes Agricultural Trust, Rothamsted Experimental Station). Mean separations were determined utilising least significant difference (lsd) at the 5% level of significance. Simple and multiple linear regressions of scatter plots were performed using Genstat and plotted using Sigmaplot (Sigmaplot for Windows version 4.01, copyright 1986-1997 SPSS Inc.).

4.3 Results

4.3.1 Shoot and inflorescence numbers

Percentage bud burst at the start of the 1997/1998 growing season was not affected by previous season's defoliation, although vines with 100% defoliation tended to have the lowest per cent bud burst (75%), but this was not significant. Likewise shoot number per vine was unaffected (Table 4.1). Both the single node and vine measurements revealed that high percentages of defoliation in the previous season (66% and 100%) significantly reduced inflorescence number per shoot compared with non-defoliated vines (Table 4.1). Single node data consistently predicted slightly higher number of inflorescences per shoot than the vine measurements. On vines, 100% defoliation reduced inflorescence number per shoot by nearly half compared with no defoliation, 0.8 inflorescences per shoot versus 1.4 respectively. At 66% defoliation inflorescence number per shoot was reduced to 1.1, while at 33% defoliation there was no significant reduction compared with no defoliation. Similarly, inflorescence number per vine was reduced more by higher intensities of defoliation in the previous season (Table 4.1).

Table 4.1 The effect of the intensity(%) of vine defoliation in the previous season on bud burst, shoots per vine, inflorescences per shoot (single node and vine) and inflorescences per vine (1997/1998).

% defoliation	100	66	33	0 (No defol.)	[1]Linear Sig.
% Bud burst	74.6 a[2]	80.9 a	81.1 a	80.1 a	NS
Shoot number per vine	18 a	19 a	19 a	18 a	NS
Inflorescence number per shoot (Single node)	0.9 a	1.3 b	1.5 bc	1.7 c	***
Inflorescence number per shoot (Vine)	0.8 a	1.1 b	1.3 bc	1.4 c	***
Inflorescence number per vine	16 a	23 b	30 c	32 c	***

[1]Linear significance at $P \leq 0.001$ (***) or not significant (NS). [2]Means within the same row with the same letter are not significantly different at $P \leq 0.05$.

4.3.2 Flower numbers, cluster size and vine yields

All defoliation intensities (33%-100%) in the previous season reduced the number of flowers formed per inflorescence compared with no defoliation (Table 4.2). At 100% and 66% defoliation inflorescence flower number was reduced to approximately 70% of no defoliation, while 33% defoliation reduced flower number to 84% of no defoliation. Berry number per cluster and cluster weight, like flower number, were reduced by previous season's defoliation (Table 4.2). At 100% and 66% defoliation, berry number and cluster weight were reduced to approximately 60% of no defoliation. Cluster weight on 33% defoliated vines was reduced to 80% of no defoliation even though berry number was not significantly reduced by 33% defoliation (Table 4.2). The significant linear response to defoliation, illustrated that more intense defoliation in the previous season resulted in fewer flowers and berries per cluster. Per cent fruitset and mean berry weight were not altered by previous season's defoliation (Table 4.2).

Total cluster number per vine reflected the response of inflorescence number to defoliation intensity, and was reduced to 40, 60 and 90% of no defoliation, for 100%, 66% and 33% defoliation respectively (Table 4.2). Vine yields were dramatically reduced by previous season's defoliation. The yield from 100% defoliated vines was

only 26% of no defoliation, which averaged 3.1kg. At 66% and 33% defoliation, yields were 42% and 75% of no defoliation respectively (Table 4.2).

Table 4.2 The effect of the intensity of vine defoliation in the previous season on flower and fruit components and vine yields (1997/1998).

% defoliation	100	66	33	0 (No defol.)	[1]Linear Sig.
Flower number per inflorescence	113 a[2]	122 ab	139 b	166 c	**
Cluster berry number	53 a	56 a	70 b	80 b	*
% Fruitset	44 a	44 a	49 a	48 a	NS
Mean berry weight (g)	0.87 a	0.89 a	0.92 a	0.98 a	NS
Cluster weight (g)	51.4 a	55.6 a	69.5 b	84.5 c	**
Total cluster number per vine	16 a	24 b	34 c	39 c	***
Vine yield (kg)	0.80 a	1.29 b	2.28 c	3.10 d	***

[1]Linear significance at $P \leq 0.001$ (***), ≤ 0.01 (**), ≤ 0.05 (*) or not significant (NS). [2]Means within the same row with the same letter are not significantly different at $P \leq 0.05$.

4.3.3 Yield component relationships

Regression analysis of floral and fruit components against vine yield revealed that there was a strong linear relationship between inflorescence number per shoot and vine yield (Figure 4.2a). Inflorescence number per shoot accounted for 80% of the variation in vine yield. Flower number per inflorescence did not relate so strongly to vine yield with a R^2 value of 0.55 however, the linear relationship was significant (P <0.001) (Figure 4.2b). Linear and multiple regression revealed that flowers per inflorescence accounted for most (74%) of the variation in berry number per cluster, however, when per cent fruitset was included in the multiple regression analysis the vast majority (96%) of variation in berry number per cluster was accounted for (Table 4.3). Total cluster number per vine related to yield in a linear manner, with nearly 80% of the variation in yield accounted for by cluster number (Table 4.3). Both clusters per vine and berries per cluster accounted for 85% of the variation in vine yield, but the addition of berry weight to the multiple regression analysis could not account for any more variation in yield (Table 4.3).

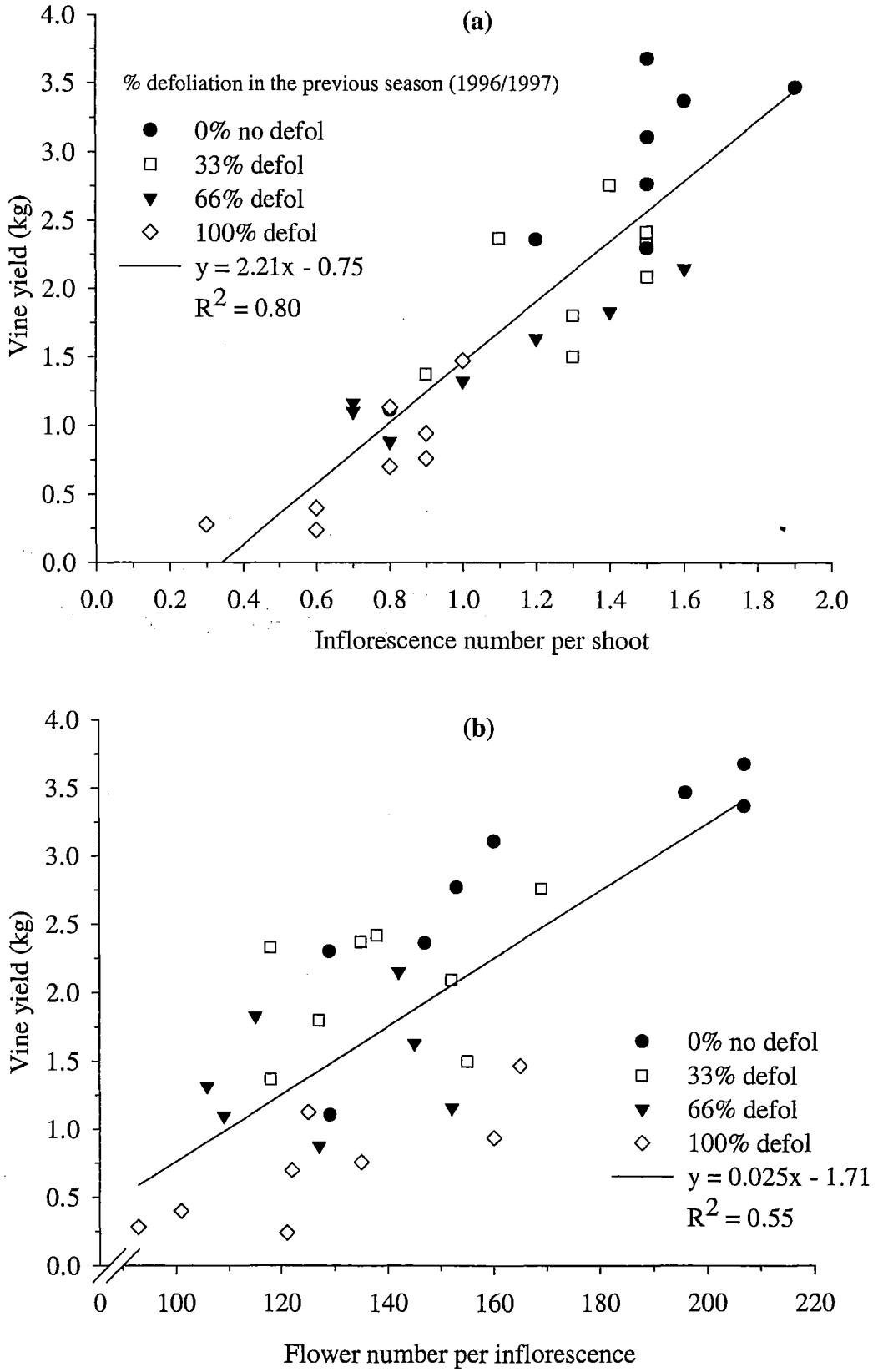

Figure 4.2 The relationship between (a) inflorescence number per shoot, (b) flower number per inflorescence, and vine yield across all defoliation treatments (1997/1998).

Table 4.3 Simple and multiple (+) linear regression coefficients of determination (R^2) and probability values from the relationships between flower and fruit components and vine yield.

Variate	R^2	*P*
Berries per cluster:		
Flowers per inflorescence	0.74	< 0.001
Flowers per inflorescence + per cent fruitset	0.96	< 0.001
Yield per vine:		
Cluster number	0.79	< 0.001
Cluster number + berry number	0.85	< 0.001
Cluster number + berry number + berry weight	0.87	< 0.001

4.3.4 Vegetative growth and vine capacity

Previous season's defoliation reduced shoot and pruning weights (Table 4.4). Shoots on 100% defoliated vines weighed 41grams which was approximately 70% of the weight of shoots on non-defoliated vines. The pruning weight of 100% defoliated vines was reduced to 65% of non-defoliated vines, which averaged 1.12kg. The Ravaz index (yield to pruning weight ratio) of non-defoliated vines was 2.8kg of fruit for every 1.0kg of prunings, this ratio fell as the intensity of previous season's defoliation increased, at 100% defoliation the ratio was 1.1kg of fruit for every 1.0kg of prunings (Table 4.4). Vine capacity, an estimate of total above ground annual dry matter production was more than halved by 100% defoliation versus no defoliation, the response to the intensity of defoliation was linear, as the intensity of defoliation increased the lower the vine capacity (Table 4.4).

Table 4.4 The effect of the intensity of vine defoliation in the previous season on vegetative growth, Ravaz index and vine capacity (1997/1998).

% defoliation	100	66	33	0 (No defol.)	[1]Linear Sig.
Shoot weight (g)	41.0 a[2]	46.7 ab	54.3 bc	61.2 c	**
Pruning weight per vine (kg)	0.72 a	0.86 ab	1.05 bc	1.12 c	***
Ravaz index	1.1 a	1.5 a	2.2 c	2.8 c	**
Vine capacity (kg)	0.60 a	0.79 a	1.15b	1.39 c	***

[1]Linear significance at $P \leq 0.001$ (***), ≤ 0.01 (**) or not significant (NS). [2]Means within the same row with the same letter are not significantly different at $P \leq 0.05$.

4.4 Discussion

4.4.1 Yield components

Results presented in Table 4.1 suggest that flower number per inflorescence may be reduced by a relatively low intensity of defoliation (33%) in the previous season before reductions in inflorescence number per shoot become apparent at higher intensities of defoliation (Table 4.1). Although flower number per inflorescence was reduced by all defoliations, the proportion of reduction over no defoliation was not as great as that of inflorescence number per shoot. At 100% defoliation flower numbers were approximately 70% of no defoliation, whereas inflorescence number was approximately 60% of no defoliation (Table 4.2). This suggests inflorescence number is more sensitive at high intensities of defoliation than flower number. Conversely flower number is more sensitive at lower intensities of defoliation.

The response of inflorescence number to previous season's defoliation on its own does not present any new information as previous studies (Candolfi-Vasconcelos and Koblet 1990, Mansfield and Howell 1981, May *et al.* 1969 and Hunter and Visser 1990) have also demonstrated reductions in inflorescence numbers in response to previous season's defoliation. For example, Hunter and Visser (1990) found that 33% defoliation of Cabernet sauvignon vines at berry set stage (same period as this trial) did not reduce inflorescence numbers, while 66% defoliation at the same time did result in reductions in inflorescence numbers (80% of no defoliation). Mansfield and Howell (1981) illustrated that 50% or 100% defoliation as late as véraison in the previous season reduced inflorescence numbers in Concord grapevines. However, Mansfield and Howell (1981) noted that the reduction in inflorescence number only occurred when a complete half of the vine was defoliated. Removing leaves on every other shoot or node did not have the same effect. May *et al.* (1969) demonstrated with Sultana vines that the negative effects of defoliation on inflorescence numbers were most evident on fully defoliated shoots. Their Sultana vines were even more sensitive than the Chardonnay vines in this trial, with reductions as low as 40% of no defoliation despite the later timing of defoliation (stage II of berry growth). Candolfi-Vasconcelos and Koblet (1990) demonstrated with

Pinot noir vines that defoliation through the removal of either main leaves or laterals in the previous season reduced inflorescence numbers to 70% of no defoliation.

The effect of previous season's vine defoliation on inflorescence flower number is not well documented, presumably because of the inherent difficulties of determining flower number. Scholefield *et al.* (1977) reported a reduction in inflorescence flower number in response to canopy death after harvest pruning of Sultana vines in the previous season. In contrast to Scholefield *et al.* (1977), Kliewer *et al.* (1988) found that defoliation of the upper parts of Sauvignon blanc canopies in the previous season resulted in small increases in inflorescence flower number. Kliewer *et al.* (1988) suggested this response might have been a consequence of improved light exposure of lower canopy leaves and the positive effect this had in turn on inflorescence initiation. Inflorescence flower number results presented in Table 4.2 do not support the Kliewer *et al.* (1988) explanation, as even a low intensity (33%) of defoliation reduced flower number. This suggests that Chardonnay vine canopies in this trial were not suffering from excessive leaf to leaf shading. Per cent fruitset in the trial presented here (Table 4.2) was not influenced by last season's defoliation and, irrespective of inflorescence flower number, the proportion of flowers that set berries was within a tight range of 44-49% (Table 4.2). The failure of previous season's defoliation to affect per cent fruitset comes as no surprise as earlier research by Caspari *et al.* (1998) suggests that per cent fruitset is strongly linked to carbohydrate supply from current season photosynthesis at bloom, rather than affected by any lingering defoliation effect from the previous season.

The reduction in cluster weight was ultimately ascribed to a reduction in berry number per cluster (Table 4.2), which in turn was primarily the consequence of a reduction in inflorescence flower number (Table 4.3). Again the reduction in berries per cluster and cluster weight in response to previous season's defoliation do not on their own present new information as earlier studies by Candolfi-Vasconcelos and Koblet (1990), Mansfield and Howell (1981) and May *et al.* (1969) have all reported reductions in berries per cluster and cluster weight in response to previous season's defoliation. Mansfield and Howell (1981) illustrated that 50% or 100% defoliation resulted in reduced yield per node (cluster weight), but the reduction only occurred when half a vine was completely defoliated. Likewise Candolfi-Vasconcelos and Koblet (1990) illustrated

that main leaf or lateral defoliation of Pinot noir vines in the previous season resulted in fewer berries per cluster and also reduced berry weight. Candolfi-Vasconcelos and Koblet (1990) were unable to explain whether the reduction in berry number was a consequence of fewer flowers or changes in per cent fruitset. In contrast, the trial presented here (Tables 4.2 and 4.3) was able to clearly demonstrate that reduction in berry number was due to decreases in flower number rather than per cent fruitset (Table 4.2) and that berry weight had no influence on changes in cluster weight (Table 4.3). Like the trial presented here, May *et al.* (1969) found that lighter clusters on Sultana vines defoliated in the previous season were the consequence of fewer berries rather than changes in berry weight. May *et al.* (1969) concluded that the decrease in berry number was probably due to a decrease in flower number rather than per cent fruitset. However, without measuring both of these parameters their conclusion can only be treated as speculation. The flower and fruit results presented in this study, in contrast to previous studies, are able to conclusively demonstrate that reductions in berries per clusters in response to previous season's defoliation are more likely to be the result of fewer flowers rather than changes in per cent fruitset.

The large reductions in vine yields shown in Table 4.2 (as little as 26% of no defoliation) were the consequence of a cumulative reduction in both inflorescence number per shoot and flower number per inflorescence. For example, 100% defoliated vines produced less than one inflorescence per shoot, compared with non-defoliated vines which produced 1.4 inflorescences per shoot (Table 4.1). On average, an individual inflorescence on 100% defoliated vines carried only 113 flowers, whereas an individual inflorescence on non-defoliated vines carried on average 166 flowers (Table 4.2). Consequently individual shoots on defoliated vines would have produced significantly fewer flowers and hence berries. Candolfi-Vasconcelos and Koblet (1990) and Mansfield and Howell (1981) illustrated that previous season's defoliation reduced flower number per inflorescence and cluster size, however, they did not recognise that the large reductions in their yields were the result of the cumulative effects of fewer inflorescences (clusters) and (flowers) berries. May *et al.* (1969) on the other hand, concluded that the large reductions in their vine yields must have been the result of both components and thus their conclusion supports the results of this trial.

The results presented here suggest that the defoliation of vines in the previous season adversely affects the processes that are responsible for the formation and/or development of inflorescences and flowers. This leads to the question; when and how does previous seasons defoliation reduce inflorescence and flower number? May 1965, Smart *et al.* 1982 and Sommer *et al.* 2000 suggest that the effects occur in the season of defoliation by affecting the process of inflorescence primordia initiation in the latent bud. To date explanations of a mechanism of effect on inflorescence initiation during the season of defoliation have centred around a restriction of carbohydrate movement into buds during the initiation period when leaves are removed or shaded (May 1965, Sommer *et al.* 2000, Thomas and Barnard 1937a). However this is yet to be proven. Alternatively it is proposed that previous season's defoliation may impede inflorescence development during the spring of the following season as consequence of the effects of defoliation on reductions in over-wintering CHO reserves.

Unlike inflorescence initiation, flowers are not formed until around bud burst in the following season (May 2000, Scholefield and Ward 1975, Srinivasan and Mullins 1981a and Thomas and Barnard 1937a). Therefore carry-over effects of defoliation in the previous season must be occurring in order to affect flower number formed per inflorescence. It is conceivable that reductions in over-wintering CHO reserves as a consequence of previous season's defoliation may limit the number of flowers formed per inflorescence, a proposition first suggested by Scholefield *et al.* (1977). Such speculation is investigated in Chapter 5.

Although yield data during the defoliation season were not available, it must also be considered that following season's yield components may also have been influenced by possible changes in crop load brought about by the direct effect of defoliation on fruit development during the defoliation season. Previous research by Candolfi-Vasconcelos and Koblet (1990) has illustrated that defoliation can result in reduced fruitset and berry size and therefore crop load. Changes in crop load can influence over-wintering CHO reserves (Weaver and McCune 1960). However in the experiment presented here, any potential crop load effect could not be determined because individual vine yield data were not available, consequently it was not possible to separate crop load and defoliation effects in regard to following season's yield components.

4.4.2 Vegetative growth and yield relationships

Even though per cent bud burst and shoot number per vine were not significantly reduced by previous season's defoliation, shoot weight was markedly reduced (Table 4.4). The lighter shoots on defoliated vines were the primary cause of reduced pruning weight. The reason why shoots were lighter remains unknown, but either decreases in shoot length or thickness were probably responsible. May *et al.* (1969) could find no negative impact of previous season's defoliation on end of season vegetative weight in Sultana vines. In contrast to May *et al.* (1969), the results of the trial presented here illustrated that previous season's defoliation not only reduced yield but also vegetative growth (pruning weight). This may be indicative of fact that there is little opportunity for compensation photosynthate production in Canterbury's short cool growing season compared with the hot long growing season of Sunraysia, Australia where May *et al.* (1969) trials were conducted. Despite reductions in pruning weight there were still major shifts in the yield to pruning weight ratios (Table 4.2) which suggest that relative to vegetative growth, reproductive growth was more adversely affected by previous season's defoliation. For example, 100% defoliated vine pruning weight was 64% of non-defoliated vines whereas yield was 26% of no defoliation (Table 4.4 and 4.2 respectively). Calculations of vine capacity (an estimate of total annual dry matter production) further illustrated that the intensity of previous season defoliation had impacted on the ability of the vine to grow (Table 4.4), this in turn suggests that little if any compensation photosynthate production occurred in the season after defoliation.

4.5 Conclusions

The results of this trial have shown that defoliation performed immediately after bloom/fruitset in the previous season does reduce inflorescence and flower number, cluster size and hence vine yields. In addition, this trial has demonstrated that reductions in cluster size are the result of smaller inflorescences that carry fewer flowers rather than changes in per cent fruitset or berry weight. Both reductions in inflorescence and flower number have cumulative affects in reducing vine yields. Of the yield components examined in this trial it appears that only inflorescence and flower number were

significantly reduced by previous season's defoliation with increasing sensitivity at the higher intensities of defoliation stress.

Vegetative growth was also shown to be reduced by increasing intensities of defoliation in the previous season and that the main factor responsible for this is a decrease in shoot weight rather than decrease in per cent bud burst and hence shoot number per vine. Major shifts in the yield to pruning weight ratio suggest that reproductive growth is more adversely affected by defoliation than vegetative growth.

Based on the findings of this experiment it is proposed that reductions in inflorescence and flower numbers due to defoliation may be the result of reduced carbohydrate supply to buds during inflorescence initiation in the current season or alternatively during inflorescence and flower formation during and after bud burst in the following season. Such propositions warrant further investigation in order to better understand the whole vine physiology behind inflorescence and flower formation. The results of this experiment suggest excessive leaf removal/defoliation (removing a third or more of the leaves per vine) shortly after bloom in the current season may have significant detrimental effects on grapevine flowering, yield and growth in the following season.

Chapter 5

The influence of the timing of vine defoliation on carbohydrate reserves, return bloom and yield

5.1 Introduction

Results presented in Chapter 4 illustrated that reductions in cluster size, in response to defoliation stress in the previous season, were the result of smaller inflorescences that carried fewer flowers. Reductions in vine yield were the consequence of not only fewer flowers per inflorescences but also of fewer inflorescences per vine. However, the physiological cause(s) for the reduction in grapevine flowering remained unknown.

Previous literature has demonstrated that vine defoliation has significant negative impacts on over-wintering trunk CHO reserves (Candolfi-Vasconcelos and Koblet 1990). Such findings confirm the hypothesis that defoliation removes a significant source of photosynthates, such that the accumulation of reserve CHO's in woody permanent parts of the grapevine is impeded. Other work has emphasised the importance of CHO reserves for both winter hardiness in cold climates and the following season's productivity (Hunter *et al.* 1995, Koblet 1996, Scholefield *et al.* 1977, Sommer *et al.* 2000), yet they have not demonstrated a clear relationship between CHO reserves and vine productivity. Based on the results of Chapter 4 and previous studies it is proposed that defoliation influences grapevine flowering primarily through its effect on carbohydrate production and partitioning to the sites of reproductive growth. The effects

of vine defoliation and CHO supply on vine flowering may be manifested in two ways. Firstly, through the direct effect of defoliation and CHO supply on inflorescence initiation as hypothesised by May (1965), Smart *et al.* (1982) and Sommer *et al.* (2000). Secondly, by the negative effects of defoliation on over-wintering CHO reserves and the subsequent influence on inflorescence development and flower formation. In addition to these propositions, reductions in over-wintering CHO reserves may even affect per cent fruitset, a situation that has not been investigated due to the belief that fruitset is solely controlled by current season's photosynthate supply (Caspari *et al.* 1998).

To examine the second and third propositions outlined above the following experimental hypotheses were developed. Firstly, vine defoliation reduces the level of over-wintering CHO reserves accumulated in both trunks and roots. Secondly, reduced levels of over-wintering CHO reserves in trunks and roots restrict the development of inflorescences and flowers during and after bud burst. Thirdly, per cent fruitset is reduced where over-wintering CHO reserves are limited. To test these hypotheses a series of experiments were carried out, which included treatments such as vine defoliation and shading to induce both photosynthate and CHO reserve stress, as well as a study of season to season variation in CHO reserves and subsequent vine productivity.

5.2 Materials and Methods

5.2.1 Defoliation

Individual 13 year old Chardonnay vines (Mendoza clone, own roots, VSP trained with two canes - ten nodes each) were defoliated at monthly intervals starting at 4 weeks post-bloom in the 1997/1998 season. The start time of 4 weeks post-bloom was chosen to ensure that defoliation did not have any negative effect on current season's fruitset and hence alter crop load. According to Candolfi-Vasconcelos and Koblet (1990) fruitset is sensitive to defoliation if it is carried out within 2-3 weeks after commencement of bloom. The defoliation of vines involved removing approximately 75% of the leaves while retaining the basal four leaves around the clusters on each shoot of the vine (Plate 5.1). The basal leaves were left to protect the clusters from sunburn and to provide some

leaf area to keep the vine alive. The monthly defoliations were performed three times, the fourth treatment was no defoliation (natural leaf fall approximately 17 weeks post-bloom). Each treatment was replicated twelve times in a randomised block design. All the shoot tips were left to grow but newly developing leaves were regularly removed to prevent any increase in leaf area. The vines were subjected to standard vineyard care – spraying for disease control and irrigation under dry conditions. Although not the focus of this experiment basic yield and fruit and vine growth data were recorded during the defoliation season (Appendix 1).

5.2.2 Carbohydrate sampling, shoot and flower measurements

At the end of the defoliation season (1997/1998) the vines were pruned back to a standard VSP system, two canes with ten nodes on each and two spurs. To obtain an early indication of the effects of defoliation on bud fertility one shoot from the prunings of each vine (suitable for laying down as a cane) was sectioned into ten single node cuttings. The single node cuttings were placed in trays of water in a heated glasshouse and left to grow, removing unwanted leaf growth occasionally. Once sufficient growth had occurred the number of inflorescences at each node was recorded.

Wood CHO samples were taken from the trunks and roots at key phenological stages throughout the following season (1998/1999) to study the recovery of CHO reserves. Trunk samples were removed from the mid section of the trunk using a small Tyovaline (Finland) trunk corer that removed a 5mm core. A section of root 1.0 to 1.5cm in diameter was removed from the root system of each vine. The wood CHO samples were handled and analysed according to the methods described in Chapter 3.1. At the start of the season (1998/1999) bud burst and shoot growth on one cane per vine were measured on a 2-weekly basis until close to bloom. A count of inflorescences on the developing shoots for the same cane was recorded. Inflorescence flower number was estimated using the methods described in Chapter 3.3. Two shoots were selected and labelled in the mid cane region (one on either cane) and branch one flower number on the inflorescences of both shoots were counted first and then the inflorescences on one of the shoots were sealed in net bags. Two shoots were selected as a precaution against the possibility that the net bags had unforeseen effects on flowering and fruitset.

5.2.3 Shading, fruit and shoot measurements

Once the flowers had been counted and net bags placed on the inflorescences, six of the twelve replicate vines for each of the defoliation treatments were completely covered in a 50% shade cloth at the start of bloom (Plate 5.2). The shade cloth remained on the vines throughout the whole bloom and early fruitset period (5 weeks) and then it was carefully removed. Temperatures in the vicinity of the inflorescences were measured using portable temperature data loggers (Gemini Tiny tag). No significant temperature differences were observed between shaded and non-shaded canopies (Figure 5.1). Prior to vine harvest all the clusters on the labelled shoots were harvested, weighed and berry number per cluster counted. Two weeks later the vines were harvested with the weight of fruit and total cluster number per vine recorded. At the end of the season (1998/1999) and once the vines had became dormant they were pruned to standard VSP system, two canes with ten nodes on each and two spurs. The prunings were weighed and a sub-sample of four randomly selected shoots per vine were measured for the following: length, weight, count of node number and shoot diameter at the eighth internode.

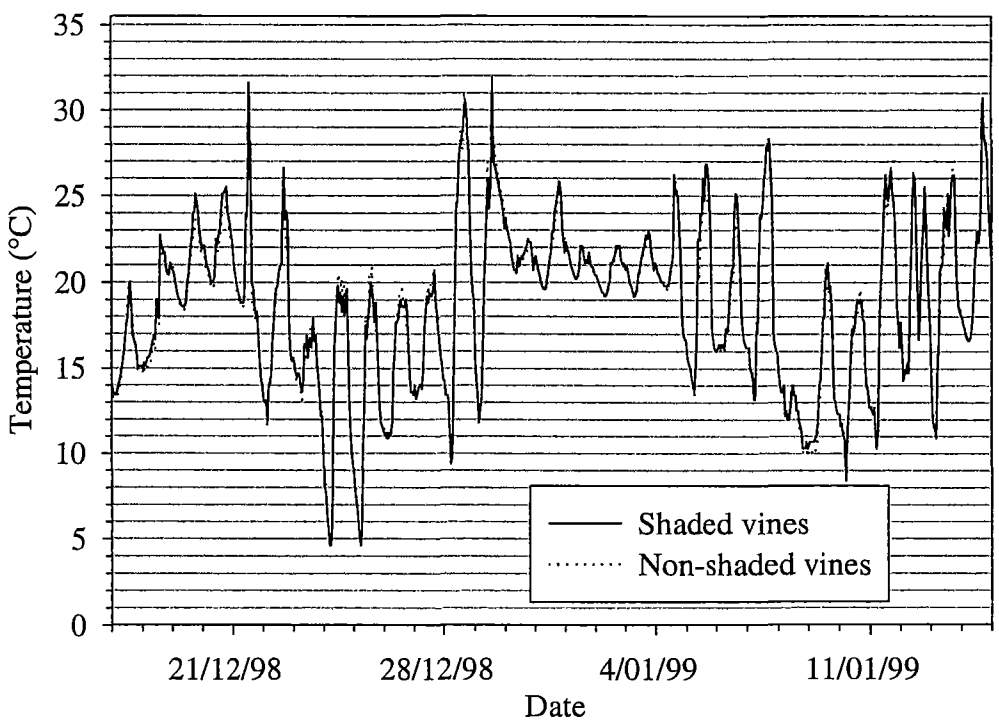

Figure 5.1 The temperature of shaded and non-shaded vine canopies from mid December 1998 to mid January 1999.

Plate 5.1 A vine with approximately 75% of its leaves removed (1997/1998).

Plate 5.2 Non-shaded vine (left) and shaded vine (right) in the following season (1998/1999).

Vine capacity (estimate of annual dry matter production) was calculated using the following formula: Vine capacity = 0.55(pruning weight) + 0.25(fruit weight), where 0.55 = percentage dry matter content of prunings and 0.25 = percentage dry matter content of fruit (Winkler *et al.* 1974). Yield to pruning weight ratios (Ravaz index) were also calculated for each treatment vine (Ravaz 1930).

5.2.4 Statistical analysis

All vine data from the four levels of defoliation x two levels of shading factorial experiment were analysed using general ANOVA testing for polynomial, main effect and interaction significance using a Genstat statistical package (Genstat 5 Release 4.1. Copyright 1997, Lawes Agricultural Trust, Rothamsted Experimental Station). Mean separations were determined utilising least significant difference (lsd) at the 5% level of significance. Simple and multiple linear and non-linear regressions of scatter plots were performed using Genstat and plotted using Sigma plot (Sigma plot for Windows version 4.01. Copyright 1986-1997 SPSS Inc.).

5.3 Results

5.3.1 Root and trunk carbohydrate reserves

Starch concentrations in the roots of vines defoliated at 4 weeks post-bloom in the previous season were only 1.5%Dwt at bud burst (29/9/1999), while for no defoliation starch was at 17%Dwt (Figure 5.2a). Defoliation at 8 weeks reduced starch concentration to 8%Dwt, approximately half that of no defoliation, while the 12 week defoliation resulted in a small but still significant decrease in starch concentration (15%Dwt). At bloom time, 80 days after bud burst (DABB), starch concentrations were at their lowest levels in all treatments, although there were still significant differences between some treatments (Figure 5.2a). By véraison, defoliation treatment effects had gone and starch concentrations in all vines ranged between 11-13%Dwt.

Root soluble sugar concentrations for the 4 week defoliation were 6.8%Dwt at bud burst (29/9/1999), which was significantly higher than all other treatments (Figure 5.2b). However, from 40 DABB right through to the end of the growing season (leaf fall - 235 DABB) there were no significant differences in sugar concentrations between the defoliation treatments. Total CHO concentrations in general reflected the response of starch to the defoliation treatments in the previous season (Figure 5.2c).

Starch concentrations in the trunks of vines defoliated 4 weeks post-bloom in the previous season were 4%Dwt at bud burst (29/9/1999), while starch concentrations in the trunks of non-defoliated vines were more than double that at 9.8%Dwt (Figure 5.3a). Eight and 12 week defoliations also significantly reduced starch concentrations, 6%Dwt and 8%Dwt respectively. At bloom (80 DABB), starch concentrations in all vines had increased to levels higher than those at bud burst, but there were still significant differences between some treatments. Treatment differences had finally gone by leaf fall, with starch concentrations ranging between 6.5%Dwt and 8.5%Dwt. Small increases in starch concentrations occurred in all vines between leaf fall and the second bud burst (28/9/1999).

Soluble sugar concentrations at bud burst were 11.7%Dwt in the trunks of 4 week defoliated vines, while in the trunks of non-defoliated vines sugar concentrations were 8.2%Dwt. All other defoliation treatments were not significantly different from no defoliation (Figure 5.3b). From 40 DABB right through to the end of the growing season (leaf fall - 235 DABB) there were only small differences in sugar concentrations between the defoliation treatments. However, large seasonal variations in sugar concentrations were evident, for example 2%Dwt between bloom and véraison and 8%Dwt to 10%Dwt at leaf fall. Total CHO concentrations, in general, reflected the response of sugar concentrations to the defoliation treatments in the previous season (Figure 5.3c).

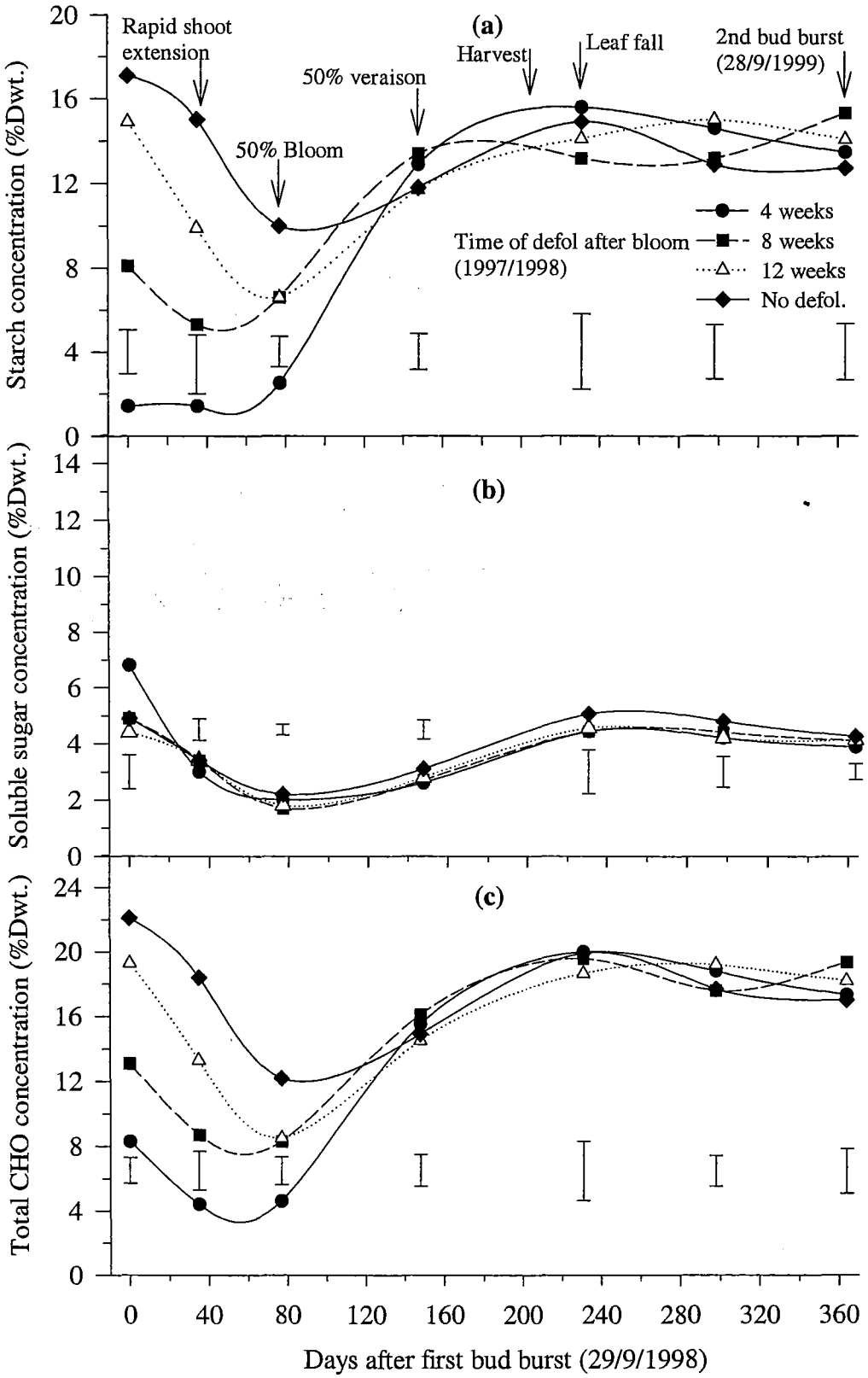

Figure 5.2 The effect of vine defoliation in the previous season on (a) starch, (b) soluble sugar and (c) total CHO concentrations in roots (1998/1999). Bars represent lsd at 5% level of significance.

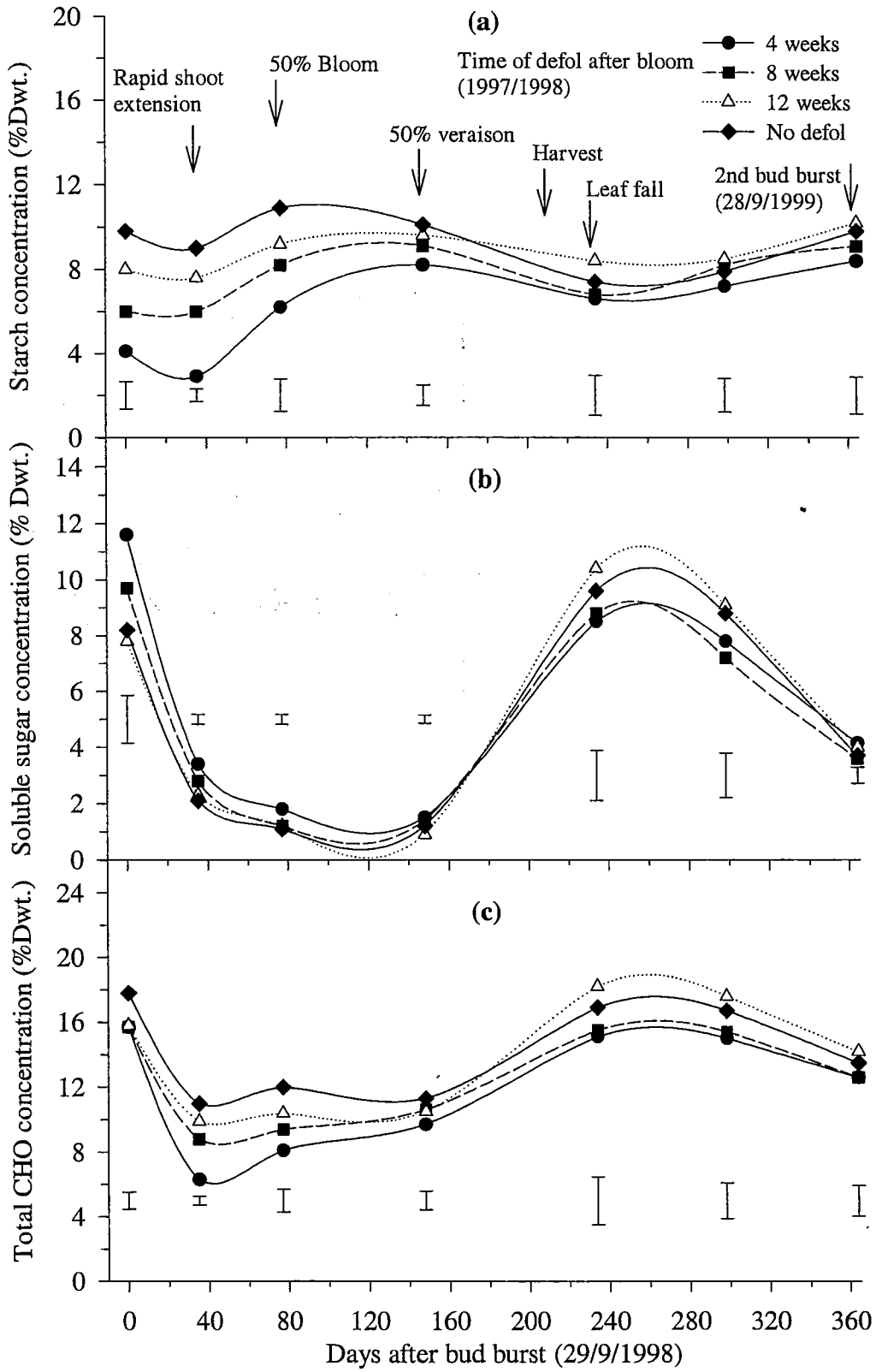

Figure 5.3 The effect of vine defoliation in the previous season on (a) starch, (b) soluble sugar and (c) total CHO concentrations in trunks (1998/1999) Bars represent lsd at 5% level of significance.

5.3.2 Flowering, fruitset and vine yield

Both the single node and vine measurements revealed that the number of inflorescences formed per shoot was significantly reduced by defoliation in the previous season (Table 5.1). The single node values were, however, consistently lower than field vine values. The response to the timing of defoliation was linear. The earlier defoliation was performed in the previous season, the lower the number of inflorescences per shoot (Table 5.1). Inflorescence number per shoot was reduced at all node positions on the 10 node cane, even where the four basal node leaves had been retained (Figure 5.4 - Single node cuttings). Node positions one and two tended to have fewer inflorescences per shoot than higher node positions on the cane (Figure 5.4). These node position effects were also observed on the vine (results not shown). Current season's shading (1998/1999) at bloom had no effect on the number of inflorescences per shoot (results not shown).

Table 5.1 The effect of defoliation in the previous season on inflorescence number per shoot (single node and vine), inflorescence branching and branch one and estimated flower number per inflorescence (1998/1999).

Time of defoliation (weeks post-bloom)	**4 weeks**	**8 weeks**	**12 weeks**	**No defol.**	**Linear Sig[1]**
Inflorescence number per shoot (Single node)	1.0 a[2]	1.3 b	1.5 bc	1.6 c	***
Inflorescence number per shoot (Vine)	1.2 a	1.6 b	1.8 b	2.2 c	***
Branch number per inflorescence	4.4 a	5.5 b	5.8 bc	6.4 c	***
Branch one flower number	24 a	35 b	37 b	43 c	***
Estimated flower number per inflorescence	152 a	221 b	235 b	270 c	***

[1]Linear significance at $P \leq 0.001$ (***). [2]Means within the same row with the same letter are not significantly different at $P \leq 0.05$.

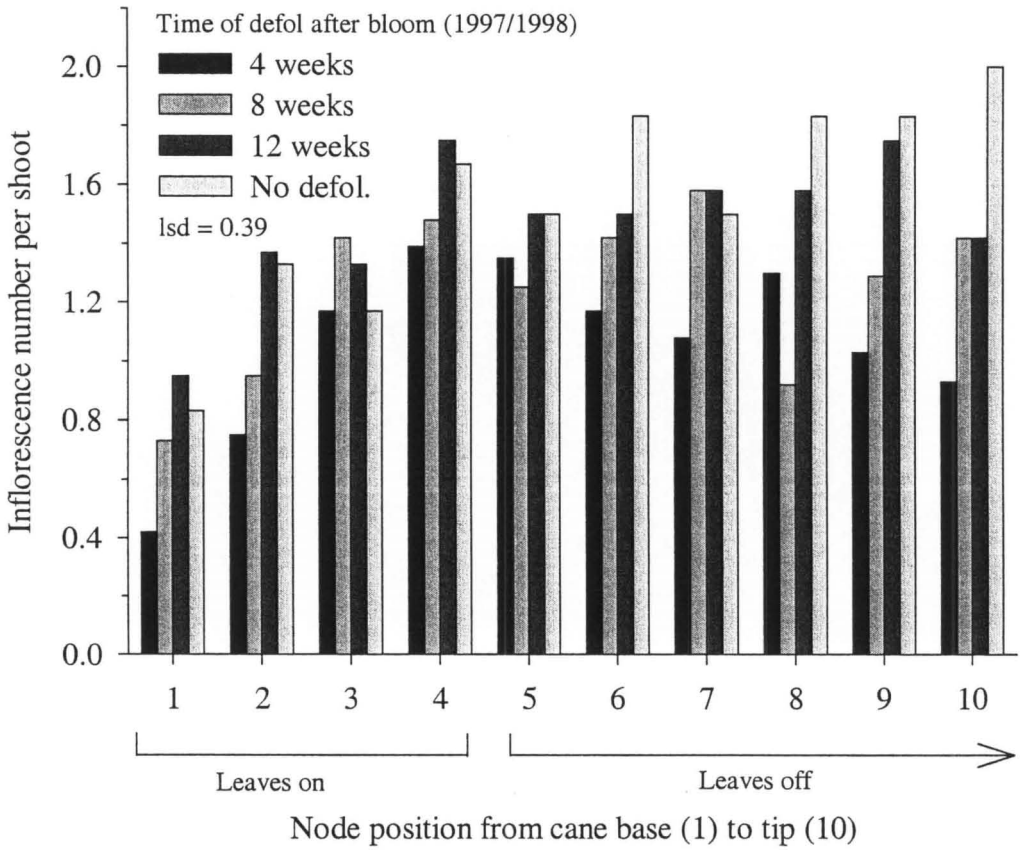

Figure 5.4 The effect of vine defoliation in the previous season on inflorescence number per shoot for node positions 1 to 10 on the cane (Single node cuttings).

Branch number per inflorescence was reduced by previous season's defoliation, for example 4 week defoliation reduced branch number to 4.4 versus 6.5 for no defoliation (Table 5.1). Branch one and estimated flower numbers per inflorescence were also reduced by defoliation. In 4 week defoliated vines an average inflorescence had approximately one hundred fewer flowers than inflorescences on non-defoliated vines. Inflorescences on vines defoliated at 8 and 12 weeks had approximately eighty more flowers than the 4 week defoliation, but still significantly fewer flowers than the no defoliation (Table 5.1). Current season's shading (1998/1999) at bloom had no effect on the estimated flower number per inflorescence (Table 5.2). The flower data presented in Tables 5.1 and 5.2 represents the average of both basal and apical inflorescences on a shoot. Analysis of inflorescence position (basal versus apical under the four defoliation treatments) revealed that apical inflorescences had approximately 40% fewer flowers than basal inflorescences and that defoliation in the previous season reduced the number

of flowers at both positions by a similar proportion (results not shown). It should be noted that presenting averaged data may have lead to an artificially inflated flower number for inflorescences on early defoliated vines. This is because there were fewer apical inflorescences on the shoots of early defoliated vines compared with later or non-defoliated vines as indicated by inflorescence number per shoot in Table 5.1.

Although the previous season's defoliation reduced the number of flowers per inflorescence (Table 5.1, Table 5.2), per cent fruitset was not significantly affected. There was however, a trend for per cent fruitset to be lower on inflorescences with more flowers in both shaded and non-shaded vines (Table 5.2). Current season's vine shading reduced the proportion of flowers that set berries, 32% fruitset for shaded vines versus 42% for non-shaded vines (Table 5.2, Plates 5.3, 5.4). Berry number per cluster, like flower number, was reduced by defoliation. Clusters on vines defoliated at 4 weeks had on average 59 berries whereas clusters on non-defoliated vines had approximately 90 berries. Berry number per cluster was further reduced on all vines by 5 weeks of 50% shade during the bloom/fruitset period (Table 5.2). Mean berry weight was not affected by previous season's defoliation or current season's shading and ranged between 0.9 and 1.3 grams. Largely as a consequence of reductions in berries per cluster, cluster weight was also reduced. The cluster weight on 4 week defoliated vines was 78g compared with no defoliation (127g); shading further reduced 4 week defoliated cluster weight to 50g (Table 5.2). Total cluster number per vine decreased the earlier defoliation treatment was imposed (linear significance, $P \leq 0.001$) (Table 5.2). This response was a reflection of the decrease in inflorescence number per shoot (Table 5.1). With reductions in both cluster weight and cluster number, vine yields were significantly reduced by previous season's defoliation and current season's shading, for example yield on 4 week defoliated vines was reduced to 1.8kg compared with 5.9kg for non-defoliated vines (Table 5.2). Shading further reduced 4 week defoliated vine yield to 1.5kg. There were no significant interactions between previous season's defoliation and current season's shading for any of the yield components examined (Table 5.2).

Table 5.2 The main effects and interactions of previous season's defoliation and current season's shading on inflorescence size, fruitset, cluster size and vine yields (1998/1999).

Treatment	50% shade				No shade				Significance		
Combination	4 wks	8 wks	12 wks	No defol.	4 wks	8 wks	12 wks	No defol.	[1]Defol.	[2]Shade	[3]Intera.
Flower number per inflorescence	156 a	217 b	230 bc	281 d	149 a	213 b	253 cd	258 d	***	NS	NS
% Fruitset	36 abc	34 ab	31 ab	27 a	45 c	46 c	40 bc	39 bc	NS	***	NS
Berry number per cluster	54 a	71 ab	72 ab	75 b	64 ab	96 c	99 c	103 c	*	**	NS
Mean berry weight (g)	0.9	1.1	1.2	1.3	1.2	1.1	1.1	1.2	NS	NS	NS
Cluster weight (g)	48.6 a	86.0 b	85.8 b	98.4 bc	78.4 ab	108.5 bc	112.3 bc	127.0 c	**	**	NS
Cluster number per vine	29 a	31 a	40 b	42 b	26 a	36 ab	41 b	51 c	***	NS	NS
Vine yield (kg)	1.48 a	2.45 bc	3.15 cd	3.70 de	1.82 ab	3.50 de	4.49 e	5.90 f	***	***	NS

[1], [2], & [3] main effects of Defoliation, Shade and Interactions respectively at $P \leq 0.05$ (*), ≤ 0.01 (**), ≤ 0.001 (***) or not significant (NS).

Plate 5.3 Low fruitset as result of 5 weeks shading (1998/1999).

Plate 5.4 High fruitset on non-shaded vines (1998/1999).

5.3.3 Yield component and carbohydrate relationships

Quadratic curves were found to best describe the relationship between inflorescence and flower numbers and yield (Figure 5.5ab). Inflorescence number per shoot accounted for 93% of the variation in vine yield (Figure 5.5a), while flower number per inflorescence accounted for 72% of the variation in yield (Figure5.5b). The regressions presented in Figure 5.5ab only used data from the non-shaded vines. Similar relationships were also found for shaded vines, although the intercepts and slopes were lower due to lighter yields. For presentation purposes the shaded group regressions were not shown on the same figure. In order to perform multiple linear regressions the raw yield data, from the non-linear relationships with floral (Figure 5.5ab) and fruit components, were converted to a linear function using logn transformation. Linear regression of transformed yield data revealed that inflorescence number per shoot and flower number per inflorescence accounted for the same percentage of variation in yield (93% and 73% respectively, Table 5.3) as the original quadratic regressions (Figure 5.5). Multiple linear regression revealed that inflorescence and flower number together accounted for 95% of the variation in yield (Table 5.3). Clusters per vine were shown by linear regression to account for 80% of the variation in yield, multiple linear regression of clusters per vine plus berries per cluster and berry weight did not account for any more variation in vine yield (Table 5.3).

Table 5.3 Simple and multiple (+) linear regression coefficients of determination and probability values from the relationships between yield and floral and fruit components.

Variate	$\mathbf{R^2}$	***P***
Logn Yield:		
Inflorescences per shoot	0.93	< 0.001
Flowers per inflorescence	0.73	< 0.001
Inflorescences per shoot + flowers per inflorescence	0.95	< 0.001
Clusters per vine	0.80	< 0.001
Clusters per vine + berries per cluster	0.82	< 0.001
Clusters per vine + berries per cluster + berry weight	0.83	< 0.001

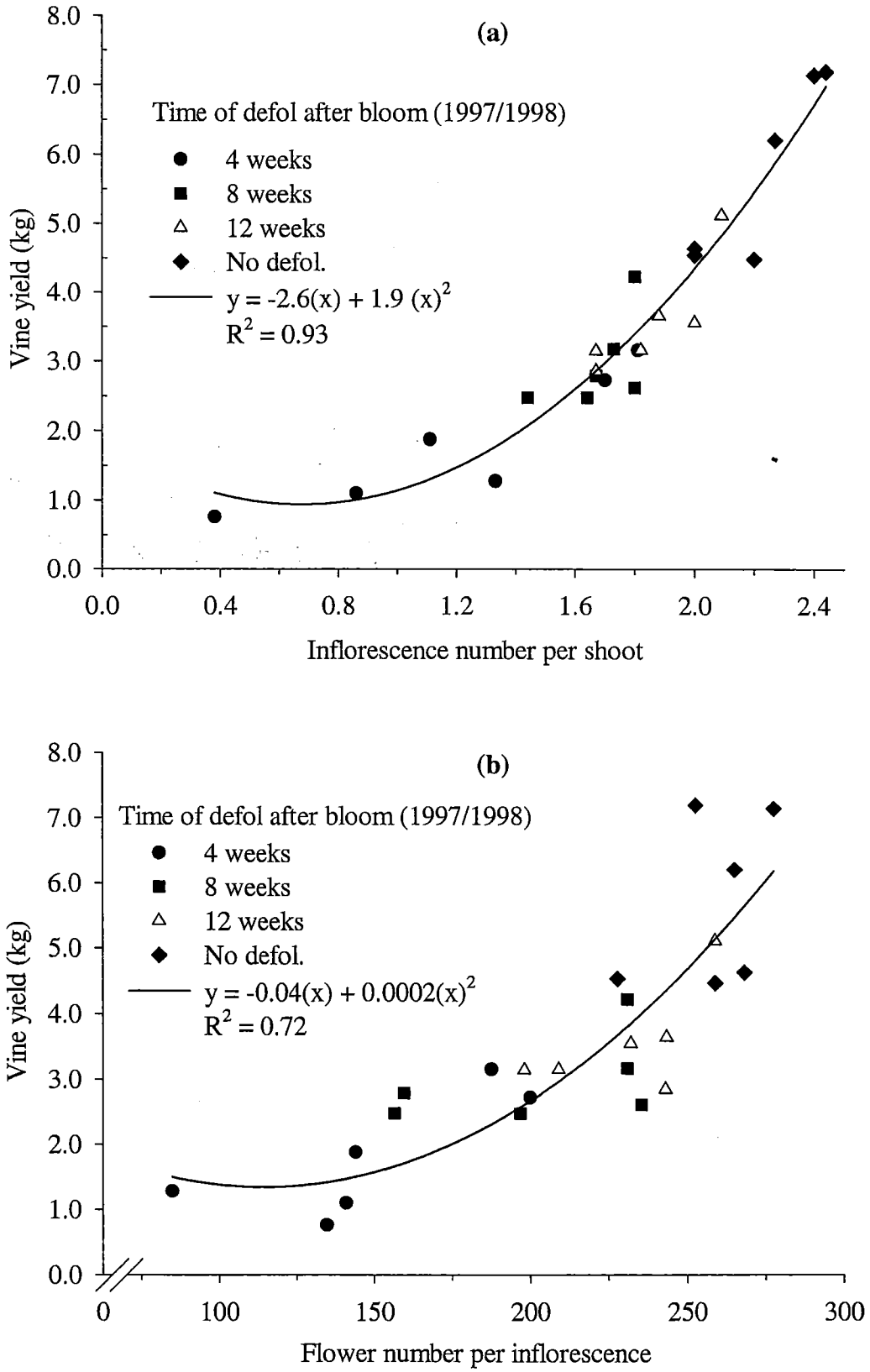

Figure 5.5 The relationship between (a) inflorescence number per shoot, (b) flower number per inflorescence and vine yield (1998/1999).

Carbohydrate and yield component data were integrated using linear and non-linear regressions. Trunk and root starch concentrations at bud burst (29/9/1998) were chosen for regression analysis because they are the most important form of reserve CHO. Trunk starch concentration was related to inflorescence number per shoot by logn regression with an R^2 of 0.71 (Figure 5.6a). Initial increases in trunk starch concentration were met with large increases in inflorescence number per shoot, but this levelled out at higher starch concentration. In contrast to the above, trunk starch concentration was related to flower number per inflorescence by a linear regression with an R^2 of 0.66 (Figure 5.6b). Likewise, trunk starch concentration related to vine yield in a linear fashion with an R^2 of 0.74 (Figure 5.6c). Root starch concentration at bud burst was also integrated with floral components and yield using the same regression analysis. Root starch concentration was related to inflorescence number per shoot by a logn regression with an R^2 of 0.76 ($P < 0.001$), while root starch concentration was related to flower number per inflorescence and vine yield by linear regressions with an R^2 of 0.70 ($P < 0.001$) and 0.66 ($P < 0.001$) respectively (scatter and regression plots not shown).

5.3.4 Vegetative Growth

Assessment of vegetative growth in the 1998/1999 season in response to the previous season's defoliation was measured. Commencement of bud burst was the same across all treatment vines, starting on 29/9/1998 (data not shown). At both 18 and 25 DABB there were no significant differences in shoot length between the four defoliation treatments (Figure 5.7). By 36 and 44 DABB non-defoliated vines had longer shoots than the 4 and 12 week defoliations, but not 8 week defoliation. At 55 DABB non-defoliated vines had shoots that were longer than all other defoliation treatments. Final shoot length and node number at the end of the growing season were not affected by the previous season's defoliation (Table 5.4). Shoot weight, however, responded to the timing of the previous season's defoliation in a linear manner, the earlier defoliation the lighter the shoot weight. Shoot diameter at the eighth internode, like shoot weight, was reduced the earlier previous season's defoliation was imposed (Table 5.4).

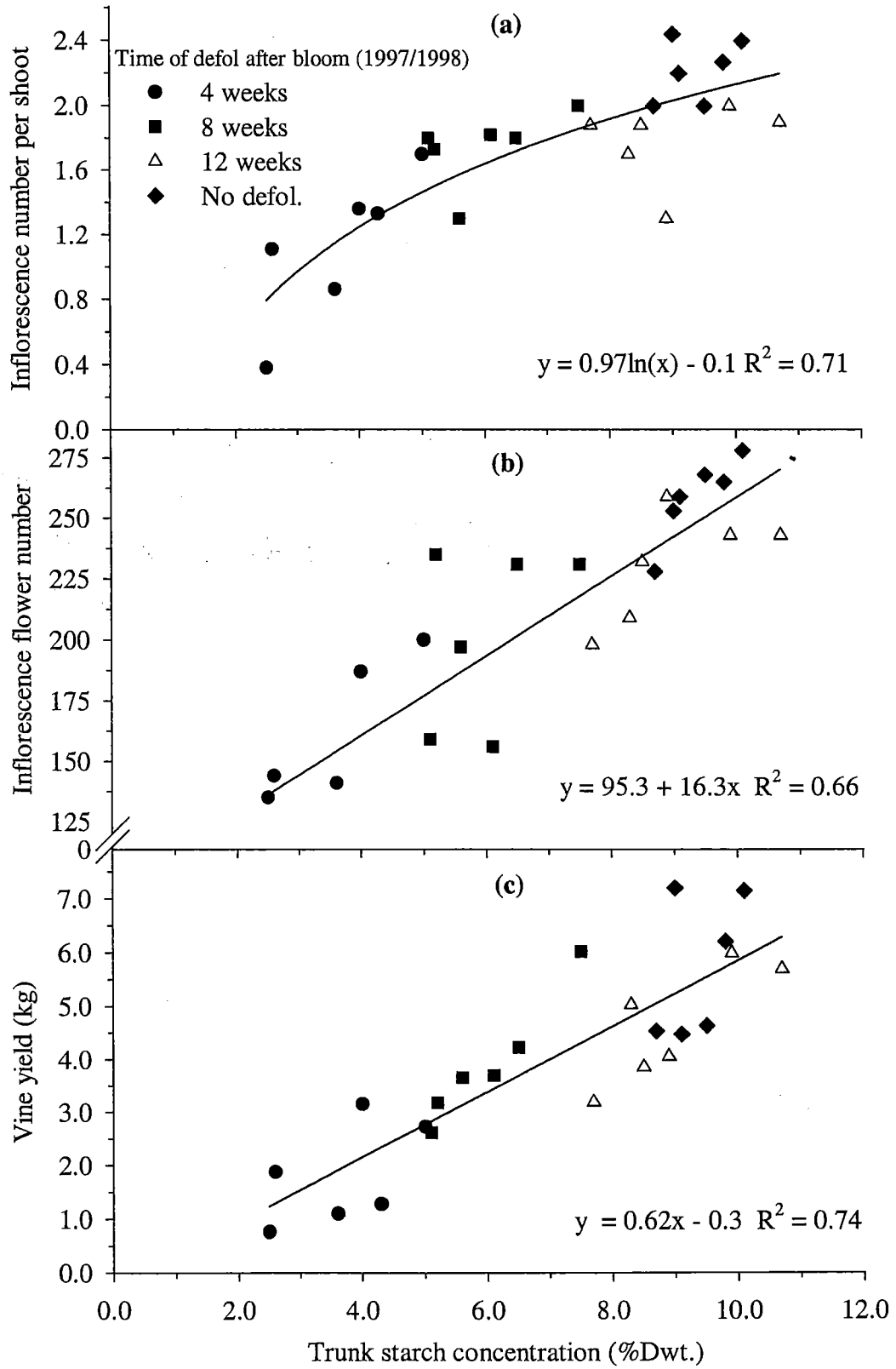

Figure 5.6 The relationship between trunk starch concentration at bud burst and (a) inflorescence number per shoot, (b) flower number per inflorescence and (c) vine yield (1998/1999).

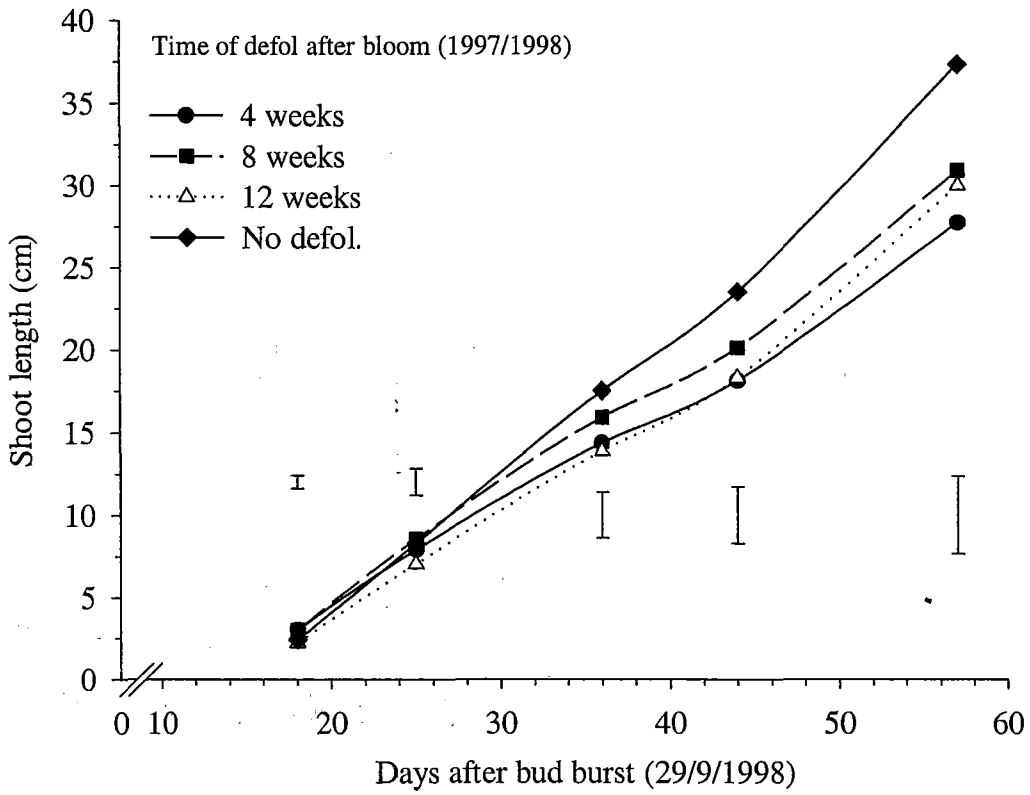

Figure 5.7 The effects of vine defoliation in the previous season on shoot length from bud burst to pre-bloom (1998/1999). Bars represent lsd at 5% level of significance.

Table 5.4 The effects of vine defoliation in the previous season on final shoot growth parameters (1998/1999).

Time of defoliation (weeks post-bloom)	**4 weeks**	**8 weeks**	**12 weeks**	**No defol.**	**Linear Sig[1]**
% Bud burst	81.8 a[2]	91.7a	85.6 a	87.9 a	NS
Shoot number per vine	22 a	24 a	23 a	23 a	NS
Shoot length (cm)	150 a	152 a	159 a	171 a	NS
Node number per shoot	22 a	23 a	20 a	22 a	NS
Shoot fresh weight (g)	62.9 a	69.6 a	82.1 b	115.0 c	***
Shoot diameter at eighth internode (mm)	7.35 a	7.80 a	8.47 b	9.60 c	***

[1]Linear significance at $P \leq 0.001$ (***) or not significant (NS) at $P \leq 0.05$. [2]Means within the same row with the same letter are not significantly different at $P \leq 0.05$.

Previous season's defoliation at either 4, 8 or 12 weeks post-bloom reduced vine pruning weight compared with no defoliation (Table 5.5). Defoliation as early as 4 weeks post-bloom more than halved pruning weight. Yield to pruning weight ratios (Ravaz index) were altered by the previous season's defoliation. Non-defoliated vines produced nearly 2kg of fruit for every 1kg of prunings (Table 5.5). The ratio was progressively reduced by earlier defoliations. Vine capacity (an estimation of total above ground annual dry matter production) was reduced more, the earlier defoliation was performed in the previous season (Table 5.5). The capacity of vines defoliated at 4 weeks was half that of non-defoliated vines. Trunk starch concentration (within non-shaded group only) at bud burst (29/9/1998) was shown by linear regression to relate strongly (R^2 0.80, $P \leq 0.001$) with vine capacity (Figure 5.8). Similarly, root starch concentration at bud burst was related with vine capacity (R^2 0.72, $P \leq 0.001$) (data not shown). Shading treatment had no effect on pruning weight, but as a consequence of reduced yield in shaded vines both yield to pruning weight ratio (Ravaz index) and vine capacity were reduced (Table 5.5).

Table 5.5 The main effects of vine defoliation in the previous season and shading in the current season on pruning weight, Ravaz index and vine capacity (1998/1999).

Time of defoliation (weeks post-bloom)	**4 weeks**	**8 weeks**	**12 weeks**	**No defol.**	**Linear Sig[1]**
Pruning fresh weight (kg/vine)	1.45 a[2]	1.63 a	2.05 b	2.47 c	***
Yield to pruning weight ratio (Ravaz index)	1.2 a	1.5 ab	1.8 bc	1.9 c	**
Vine capacity (kg)	1.24 a	1.50 a	2.06 b	2.44 c	***

Shade treatment	**50% shade**	**No shade**
Pruning fresh weight (kg/vine)	1.94 a	1.87 a
Yield to pruning weight ratio (Ravaz index)	1.3 a	1.9 b
Vine capacity (kg)	1.68 a	1.94 b

[1] Linear significance at $P \leq 0.001$ (***), ≤ 0.01 (**). [2]Means within the same row with the same letter are not significantly different at $P \leq 0.05$.

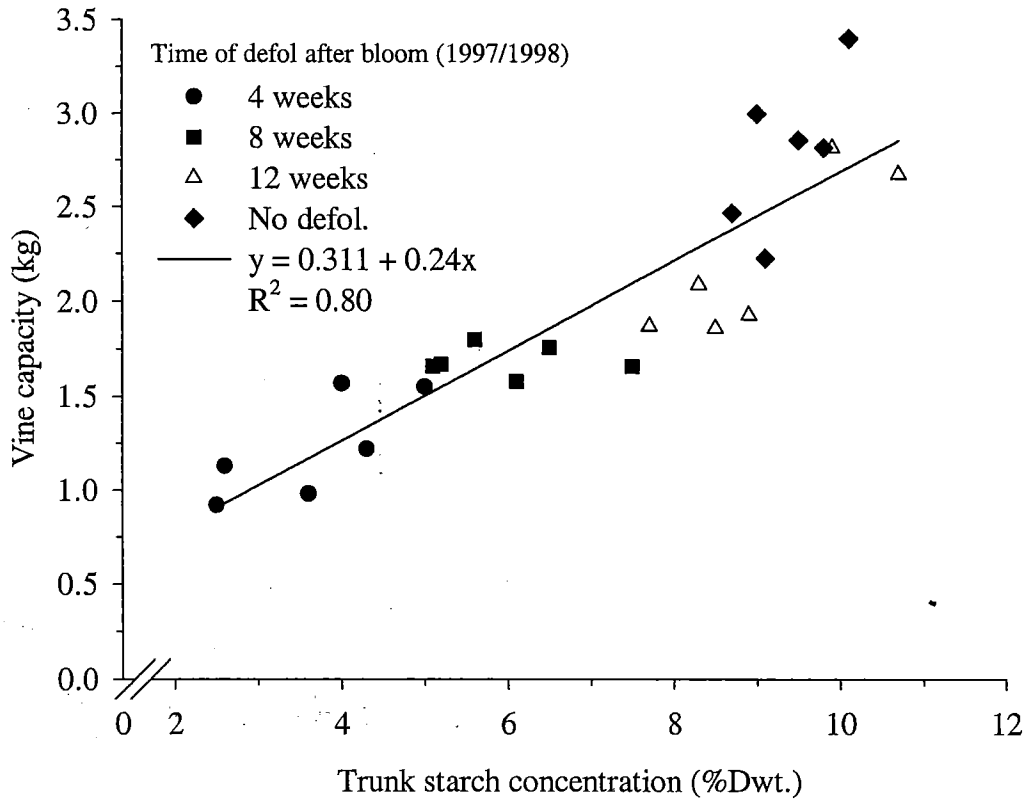

Figure 5.8 The linear relationship between trunk starch concentrations at bud burst and vine capacity (non shaded group only) (1998/1999).

5.3.5 Defoliation and shading carry-over effects in 1999/2000

5.3.5.1 Carbohydrate reserves

At the start of the second season (1999/2000) the effects of defoliation on root and trunk CHO reserves had gone (see Figures 5.2 and 5.3 - 2nd bud burst 29/9/1999). The shading treatment at bloom in the previous season (1998/1999) did however, reduce trunk and root CHO reserves compared with non-shaded vines (Table 5.6). Trunk starch concentration in shaded vines was 7.2%Dwt, versus 8.8%Dwt in non-shaded vines, while root starch concentration was 11.0%Dwt for shaded vines versus 13.9%Dwt for non-shaded vines. As a consequence of reduced starch concentration, total CHO concentration in the roots and trunks of shaded vines was lower than non-shaded vines (Table 5.6).

Table 5.6 The effects of vine shading in the previous season on trunk and root CHO reserves at bud burst (29/9/1999).

Shade Treatment	50% Shade	No shade
Trunk soluble sugar (%Dwt.)	4.0 a	3.9 a
Trunk starch (%Dwt.)	7.2 a[1]	8.8 b
Trunk total CHO (%Dwt.)	11.2 a	12.7 b
Root soluble sugar (%Dwt.)	4.1 a	4.0 a
Root starch (%Dwt.)	11.0 a	13.9 b
Root total CHO (%Dwt.)	15.1 a	17.9 b

[1]Means within the same row with the same letter are not significantly different at $P \leq 0.05$.

5.3.5.2 Flowering, fruiting and yields

The 4 week defoliation two seasons previously reduced inflorescence number per shoot to 1.5 from 1.8 for no defoliation. (Table 5.7). Inflorescence branching and estimated flower number were also lower in 4 week defoliated vines compared with no defoliation (Table 5.7). However, across all defoliations a linear response was evident with earlier defoliations having fewer inflorescences and flowers than later defoliations (Table 5.7). Per cent fruitset was lower in 4 and 8 week defoliations, 14-16% versus 22% for no defoliation, likewise, there were fewer berries per cluster for 4 and 8 week defoliations (Table 5.7). As a consequence of fewer berries per cluster, clusters were lighter, the earlier the defoliation treatment was imposed. Cluster number per vine and yield, like many of the other contributing yield components, were lower in vines defoliated at 4 and 8 weeks post-bloom two seasons previously compared with no defoliation (Table 5.7).

Previous season's shading reduced the number of inflorescences per shoot to 1.5 from 1.9 for non-shaded vines and also reduced inflorescence branching and estimated flower number (Table 5.8). Per cent fruitset was not significantly altered by previous season's shading, but as a consequence of fewer flowers, berries per cluster and cluster weight were lower in shaded vines. Lighter clusters and fewer of them per vine contributed to a 42% reduction in vine yield for shaded vines (Table 5.8).

Table 5.7 The effect of vine defoliation two seasons previously on inflorescence and flower numbers, cluster size and vine yields (1999/2000).

Time of defoliation (weeks post-bloom)	4 weeks	8 weeks	12 weeks	No defol.	Linear Sig[1]
Inflorescence number per shoot	1.5 a[2]	1.6 ab	1.8 b	1.8 b	*
Branch number per inflorescence	5.0 a	6.0 ab	6.2 ab	6.3 b	*
Estimated flower number per inflorescence	163 a	178 ab	190 b	192 b	*
% Fruitset	13.9 a	16.3 ab	20.6 bc	21.6 c	**
Berry number per cluster	25 a	30 ab	40 bc	41 c	**
Mean berry weight (g)	0.79 a	0.78 a	0.77 a	0.77 a	NS
Cluster weight (g)	21.7 a	25.7 ab	33.3 b	34.1 b	**
Cluster number per vine	28 a	31 ab	33 ab	37 b	*
Vine yield (kg)	0.63 a	0.86 ab	1.10 bc	1.32 c	**

[1] Linear significance at $P \leq 0.05$ (*),≤ 0.01 (**) or not significant (NS). [2]Means within the same row with the same letter are not significantly different at $P \leq 0.05$.

Table 5.8 The effect of vine shading in the previous season on inflorescence and flower numbers, cluster size and vine yields (1999/2000).

Shade Treatment	50% Shade	No shade
Inflorescence number per shoot	1.5 a[1]	1.9 b
Branch number per inflorescence	5.0 a	7.0 b
Flower number per inflorescence	176 a	200 b
% Fruitset	16.0 a	20.1 a
Berry number per cluster	28 a	41 b
Mean berry weight (g)	0.79 a	0.76 a
Cluster weight (g)	24.0 a	33.4 b
Cluster number per vine	29 a	35 b
Vine yield (kg)	0.72 a	1.24 b

[1]Means within the same row with the same letter are not significantly different at $P \leq 0.05$.

5.3.5.3 Carbohydrate and yield component relationships

In order to examine the relationship between CHO reserves and floral components in the shaded and non-shaded vines the lingering defoliation effect (Table 5.7) had to be removed by excluding all defoliation treatments. Therefore only non-defoliated shaded and non-shaded vines were used in the regression analyses. Results from the regression analyses revealed linear relationships between trunk starch concentration at bud burst and inflorescence number per shoot (Figure 5.9a) and flower number per inflorescence (Figure 5.9b).

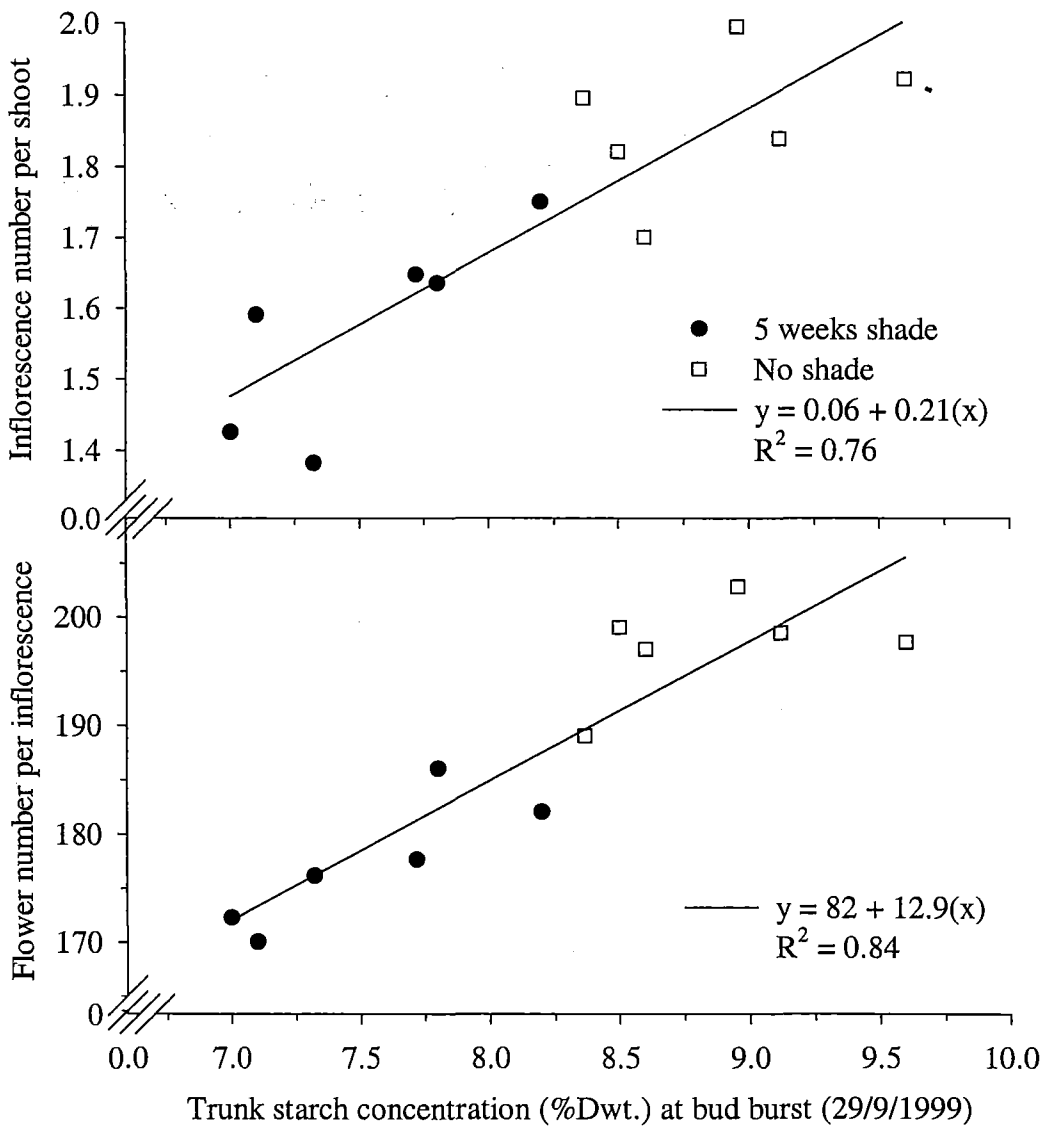

Figure 5.9 The relationship between trunk starch concentration at bud burst and (a) inflorescence number per shoot and (b) flower number per inflorescence across shaded and non-shaded vines.

5.3.6 Defoliation and shading carry-over effects in 2000/2001

5.3.6.1 Carbohydrate reserves

At bud burst of the third season after original defoliation, there was no effect of the defoliation treatment on trunk starch, soluble sugar or total CHO concentrations (Table 5.9). Starch and total CHO concentrations in the trunks of vines shaded two seasons previously, however, were significantly higher than the non-shaded vines. There were no differences in soluble sugar concentrations (Table 5.9). Root CHO reserves were not measured.

Table 5.9 The effect of vine defoliation three seasons previously and vine shading two seasons previously on trunk CHO reserves at bud burst (27/9/2000).

Time of defoliation (weeks post-bloom)	**4 weeks**	**8 weeks**	**12 weeks**	**No defol.**	**Linear Sig[1]**
Trunk soluble sugar (%Dwt.)	2.4 a	2.4 a	2.4 a	2.7 a	NS
Trunk starch (%Dwt.)	9.7 a[2]	9.4 a	10.1 a	9.5 a	NS
Trunk total CHO (%Dwt.)	12.1 a	11.8 a	12.5 a	12.2 a	NS

Shade treatment	**50% shade**	**No shade**
Trunk soluble sugar (%Dwt.)	2.4 a	2.5 a
Trunk starch (%Dwt.)	10.4 a	8.9 b
Trunk total CHO (%Dwt.)	12.9 a	11.4 b

[1] Linear significance at $P \leq 0.05$ not significant (NS). [2]Means within the same row with the same letter are not significantly different at $P \leq 0.05$.

5.3.6.2 Flowering, fruiting and yields

Throughout the third growing season (2000/2001) following defoliation there was no evidence of a defoliation effect on inflorescence and flower numbers or per cent fruitset (Table 5.10). Likewise there was no defoliation effect on berries per cluster, cluster weight or vine yield (Table 5.10).

Table 5.10 The effect of vine defoliation three seasons previously on inflorescence and flower numbers, cluster size and vine yields (2000/2001).

Time of defoliation (weeks post-bloom)	4 weeks	8 weeks	12 weeks	No defol.	Linear Sig[1]
Inflorescences per shoot	1.6 a[2]	1.5 a	1.5 a	1.4 a	NS
Estimated flowers per inflorescence	112 a	107 a	120 a	110 a	NS
% Fruitset	36.1 a	36.3 a	38.7 a	34.0 a	NS
Berries per cluster	43 a	36 a	44 a	37 a	NS
Mean berry weight (g)	1.00 a	0.92 a	1.02 a	1.05 a	NS
Cluster weight (g)	45.6 a	35.9 a	49.8 a	40.8 a	NS
Clusters per vine	26 a	26 a	24 a	27 a	NS
Vine yield (kg)	1.10 a	0.95 a	1.19 a	1.10 a	NS

[1] Linear significance at $P \leq 0.05$ not significant (NS). [2]Means within the same row with the same letter are not significantly different at $P \leq 0.05$.

Despite elevated trunk starch concentrations in vines shaded two seasons previously there were no effects on yield components in the 2000/2001 season (Table 5.11).

Table 5.11 The effect of vine shading two seasons previously on inflorescence and flower numbers, cluster size and vine yields (2000/2001).

Shade Treatment	50% Shade	No shade
Inflorescence number per shoot	1.5 a[1]	1.5 a
Estimated flower number per inflorescence	117 a	110 a
% Fruitset	39.0 a	31.0 a
Berry number per cluster	44 a	37 a
Berry weight (g)	1.02 a	1.00 a
Cluster weight (g)	48.2 a	40.7 a
Clusters per vine	27 a	25 a
Vine yield (kg)	1.28 a	1.10 a

[1]Means within the same row with the same letter are not significantly different at $P \leq 0.05$.

5.4 Discussion

5.4.1 Carbohydrate reserves

Root CHO reserves, in particular starch concentrations, were more severely affected by previous season's defoliation than the trunk CHO reserves, with starch concentrations as low as 1.5%Dwt at the end of the dormant period (Figure 5.2a). The response of over-wintering root and trunk CHO reserves to the timing of previous season's defoliation (Figures 5.2a and 5.3a) illustrated that the greater the period of time without leaf area the lower the starch concentration. Such findings provide evidence to confirm the proposition that defoliation removes a significant source of photosynthate for CHO reserve accumulation in not only trunks (Candolfi-Vasconcelos and Koblet 1990), but also roots (Figure 5.2). However, Koblet *et al.* (1993) have demonstrated that reductions in leaf area (photosynthate supply) may not be sole cause of a reductions in over-wintering CHO reserves. Koblet *et al.* (1993) found that ripening fruit on highly stressed vines (defoliation) initiated the remobilisation of CHO reserves to allow for fruit maturation. This phenomenon was confirmed by Candolfi-Vasconcelos *et al.* (1994) using ^{14}C- labelled CHO reserves. When vines were defoliated during early fruit development a much higher occurrence of ^{14}C- carbon from CHO reserves reappeared in ripening fruit compared with fruit on vines that were defoliated at a late stage of ripening or not at all. With such an alteration in CHO reserves demonstrated in the trial presented here, it is possible that the very low concentrations of starch in roots and trunks of the earliest defoliation (4 weeks) (Figures 5.2 an 5.3) were the result of not only reduced photosynthate supply, but also of the remobilisation of CHO reserves to ripening fruits in the season of defoliation. Some suggestive evidence of this is shown by the fact that berry soluble solids in the fruit from 4 week defoliated vines was not reduced compared with later defoliations in the season of defoliation treatment (1997/1998) (Appendix 1).

The monitoring of root and trunk CHO concentrations at key phenological stages throughout the season following defoliation (1998/1999) provided an opportunity to observe the depletion and subsequent accumulation of CHO reserves in both trunks and roots. Trunk and root CHO results indicated that the depletion of CHO reserves

following bud burst could be detected by reductions in starch concentrations over time, particularly in the roots (Figure 6.2b). Numerous studies using ^{14}C- labelled carbon have illustrated that roots and trunks are organs of reserve CHO accumulation and storage and that these reserves are utilised in the development of new shoots and inflorescences in the following spring (Koblet and Perret 1980, 1982, Murisier and Aerny 1994, Scholefield *et al.* 1978, Yang and Hori 1979, 1980, Yang *et al.* 1980). Results presented here (Figures 5.2 and 5.3) reinforce the importance of both roots and trunk as CHO reserve storage organs.

Minimum root CHO concentrations occurred at, or around, bloom time for all defoliations even though treatment differences were still evident (Figure 5.2abc), an observation supported by the work of Mullins *et al.* (1992), Williams (1996), and Winkler and Williams (1945). Recovery in terms of a disappearance of defoliation effect on root CHO's was accomplished by higher rates of starch accumulation in early defoliations versus no defoliation during the bloom to véraison period (see slope of lines in Figure 5.2a). The increased rate of starch concentration in early defoliations versus no defoliation was most probably the consequence of early defoliated vines carrying considerably lighter crop loads (Table 5.2). Despite the large differences in crop loads (Table 5.2) root CHO reserves in all vines fully recovered by véraison of the 1998/1999 season (Figure 5.2). In contrast to roots, trunk CHO concentrations did not fully recover from defoliation effects until leaf fall of the 1998/1999 season (Figure 5.3abc).

Minimal increases in root and trunk CHO's occurred after harvest, because leaf fall commenced at approximately the same time as harvest (Figures 5.2 and 5.3) and hence the supply of photosynthates to reserve organs ceased. In warmer climates like California, where leaf photosynthesis continues after harvest, Williams (1996) has shown that starch and sugar accumulation continued in the trunks of Thompson Seedless vines. Additional periods of photosynthate production and CHO accumulation may account for the higher productivity of grapevines in warm climates. Higher productivity may be the consequence of the effects of higher levels of CHO reserves on enhanced flowering and therefore yields in the subsequent season.

5.4.2 Yield components

The two most notable yield component responses were reductions in inflorescence number per shoot and flower number per inflorescence, when earlier defoliation was carried out in the previous season compared with no defoliation (Table 5.1 and 5.2). The earliest defoliation at 4 weeks post-bloom in the previous season effectively halved the number of inflorescences per shoot and halved the number of flowers per inflorescence. The reduction in inflorescences per shoot occurred at all node positions on the cane (Figure 5.4), even where the four basal node leaves had been retained in the previous season. This result suggests that a whole vine reaction, in terms of inflorescence development, was induced in response to 75% vine defoliation in the previous season. The reduction in fruitfulness (inflorescences per shoot) was consistent with the results in Chapter 4 (Table 4.1), where increased intensity of defoliation in the previous season reduced fruitfulness, and also with previous studies by Hunter and Visser (1990) and Mansfield and Howell (1981). Research by Candolfi-Vasconcelos and Koblet (1990) revealed conflicting reports on the effect of the timing of previous season's defoliation on inflorescences per shoot. Candolfi-Vasconcelos and Koblet (1990) could find no significant reduction in the number of inflorescences per shoot on Pinot noir vines that were defoliated either 1 or 6 weeks post-bloom in the previous season. Yet, in another experiment where vines were defoliated 0, 2, 4 or 6 weeks post-bloom Candolfi-Vasconcelos and Koblet (1990) found that the number of inflorescences per shoot was half that of no defoliation. However, one has to be cautious about the results of their second experiment because they were based solely on single node cutting measurements rather than vine measurements.

It is possible that Candolfi-Vasconcelos and Koblet (1990) single node data may not have accurately represented vine responses. Single node results presented here (Table 5.1) and in Chapter 4 (Table 4.1) suggest that single node cuttings may not accurately predict inflorescence number per shoot. In Chapter 4 (Table 4.1) single node cuttings consistently over predicted inflorescence number per shoot, while in this chapter (Table 5.1) they consistently under predicted. Even though single node cutting results revealed significant defoliation effects, such discrepancies illustrate that single node cuttings may not accurately reflect vine measurements, and therefore the information they provide

comes with a degree of uncertainty or error. As long as the degree of error can be tolerated by the experimenter single node cuttings still have the potential to provide very useful information on bud fruitfulness. Possible reasons for the discrepancy between single node and vine measurements include; firstly sampling canes that were not representative of the canes laid down at pruning. Every effort was made to select canes for single node cuttings that were identical to canes laid down at pruning in terms of diameter. Secondly, the small size of individual single node cuttings meant shoot growth and development was restricted such that the full complement of inflorescence primordia per bud did not always develop into visible inflorescences that could be recognised, counted and recorded.

The decrease in flower number per inflorescence in response to early defoliation in the previous season was associated with a decrease in the number of branches per inflorescence (Table 5.1). However, reductions in the number of flowers on branch one (Table 5.1) suggest that decreases in flower number per inflorescence were not solely attributed to reduced branching, but also to a reduction in the number of flowers formed on individual branches. May (2000) has illustrated that the number of primary branches does not always account for differences in flowers per inflorescence. In some instances May (2000) found that differences in flower number were attributed to changes in the level of secondary branching rather than primary branching. Therefore it is probable that fewer flowers on branch one (Table 5.1) were the consequence of less secondary and/or tertiary branching. This however cannot be confirmed as secondary and tertiary branching were not measured in this experiment.

Per cent fruitset was not reduced by previous season's defoliation (Table 5.2). This result suggests that reductions in the level of over-wintering CHO reserves have no direct effect on per cent fruitset, but may affect per cent fruitset indirectly through their influence on inflorescence flower number. Although not statistically significant, early defoliated vines that had fewer flowers per inflorescence consistently had higher per cent fruitset than inflorescences with more flowers on non-defoliated vines (Table 5.2). Similar inverse relationships between flower number per inflorescence and per cent fruitset have been reported by Vasconcelos and Castagnoli (2000) (Pinot noir), Keller *et al.* (2001) (Müller Thurgau) and Coombe (1962) (Muscat of Alexandra and Grenache).

Vasconcelos and Castagnoli (2000) suggest this phenomenon is a compensation mechanism that allows for an additional opportunity to regulate crop depending on the availability of resources.

Per cent fruitset, was however, reduced by 5 weeks of vine shading during the bloom/fruitset period (Table 5.2). The reduction in fruitset occurred across all defoliation treatments, irrespective of the number of flowers per inflorescence. The reduction in fruitset is consistent with previous studies. Ferree *et al.* (2001) for example, illustrated with container grown French hybrid grapes that whole vine shading for short (5 days) or long (5 weeks) periods of time reduced fruitset and that increasing intensities of shading resulted in linear decreases in fruitset. Earlier work with vinifera cultivars in the field (Nuno 1993, Ollat 1993) and in controlled environments (Roubelakis and Kliewer 1976) have also shown reduced fruitset in the presence of heavy shading. General consensus among these studies states that shading reduces fruitset by reducing leaf photosynthesis and thus CHO supply to inflorescences. Ferree *et al.* (2001) demonstrated that 50% shading resulted in 25% decrease in net photosynthesis of leaves, while Cartechini and Pallioti (1995) illustrated that the net photosynthesis and starch concentrations of leaves grown under 60% and 30% of full sunlight were reduced by more than half at bloom. Keller and Koblet (1994) demonstrated, using different light levels to vary photosynthesis, that decreased carbon availability was responsible for a reduction in fruitset and also increases in inflorescence necrosis. Caspari *et al.* (1998) found with Sauvignon blanc that linear decreases in fruitset were closely related to reductions in photosynthetic leaf area per shoot. The above studies show that the level of photosynthate supply, whether controlled by the rate of photosynthesis or leaf area, is a critical factor for fruitset. Therefore it is reasonable to conclude that the reduction in fruitset in response to 5 weeks of 50% shade in this experiment (Table 5.2) was caused by decreases in photosynthate supply to inflorescences. Based on this reasoning the findings presented here (Table 5.2) contribute to the understanding that per cent fruitset is controlled by current photosynthate supply rather than by CHO sourced from reserves.

The reduction in berry number per cluster and hence cluster weight in response to previous season's vine defoliation was primarily the consequence of fewer flowers per inflorescence (Table 5.2). The small changes in fruitset noted between defoliations

(Table 5.2) were not great enough to offset the flower number effect on berry number per cluster. Candolfi-Vasconcelos and Koblet (1990) have also found that previous season's vine defoliation 1 or 6 weeks post-bloom reduced berry number per cluster and thus cluster weight in the following season, however they were unable illustrate whether the reduction in berries per cluster was a consequence of either fewer flowers per inflorescence or reduced per cent fruitset. In contrast the results of the experiments presented here (Table 5.2) were able to clearly show that a reduction in flowers per inflorescence was the main mechanism for reducing berries per cluster and thus cluster weight. Results presented in Chapter 4 (Table 4.2 and 4.3) also confirmed this. The reduction in cluster weight and cluster number per vine (as a consequence of fewer inflorescences per shoot – Table 5.1) were cumulative in reducing vine yields (Table 5.2) and thus explain why yield was reduced so much in defoliated vines. Individually, either inflorescences per shoot or flowers per inflorescence accounted for a large proportion of the variation in vine yields (Figure 5.5ab). The fact that multiple regression of inflorescences + flowers did not account for more of the variation in yield (Table 5.3) was because neither inflorescence or flower number were independent of each other, that is, as inflorescence number increased so did flower number (Figure 5.5ab). Fruit component regressions further illustrated that a high proportion of the variation in the yields of defoliated vines was due to changes in clusters per vine (inflorescences) and berries per cluster (flowers) (Table 5.3). Current season's vine shading however, simply depressed vine yield by reducing per cent fruitset and hence berries per cluster and cluster weight (Table 5.2).

The potential influence of previous season's crop load (during the defoliation season) on the yield components discussed above can be discounted because previous season's yields were not significantly influenced by the defoliation treatments (Appendix 1).

5.4.3 Vegetative growth and vine capacity

Although there were reductions in root and trunk CHO reserves, per cent bud burst and therefore shoot number per vine were not reduced by previous season's defoliation (Table 5.4). Similar observations were also clearly evident in the intensity of defoliation trial described in Chapter 4 (Table 4.1). However, early season shoot length was reduced

by most defoliations during the period of 44 to 56 DABB (Figure 5.7). Shoot length measurements after this time period were discontinued to avoid damage to flowering inflorescences. Measurement of end of season shoot length and nodes per shoot revealed that they were unaffected by the previous season's defoliation (Table 5.4). The conflicting shoot length results presented in Figure 5.7 and Table 5.4 suggest that at some stage later on in the growing season the shorter shoots on defoliated vines caught up to shoots on non-defoliated vines. Shoot growth is the product of both stored CHO's (early season) and photosynthesis (later in the season) (Koblet and Perret 1980, 1982, Murisier and Aerny 1994, Scholefield *et al.* 1978, Yang and Hori 1979, 1980, Yang *et al.* 1980). Therefore these results suggest that early season shoot growth was impeded by reduced CHO reserves in trunks and roots, but possible compensatory shoot growth from current photosynthate may have occurred later on such that shoot lengths were similar across all defoliations by the end of the 1998/1999 growing season.

In contrast to shoot length and node number, shoot weight was reduced the earlier defoliation was imposed in the previous growing season (Table 5.4). The principal mechanism behind reduced shoot weight was a decrease in shoot diameter (Table 5.4), reducing shoot volume and hence shoot weight. To the viticulturist a reduction in shoot diameter (thickness) is interpreted as a reduction in shoot vigour. Therefore, the reductions in pruning weight per vine across defoliations (Table 5.5) can be attributed to reduced shoot vigour (weight) (Table 5.4). The shoot weight/diameter results suggest a close link between available CHO reserves at the start of the growing season and shoot vigour. Shoot diameter is to a certain extent a function of lateral cell division and subsequent expansion (Srinivasin and Mullins 1981a). It is quite conceivable that reduced CHO reserves restricted the amount of lateral cell division and expansion, which in turn could account for thinner lighter shoots.

Assessment of total vine annual dry matter production (vine capacity) revealed that, like both fresh vine yield and pruning weights, dry matter production was reduced by previous season's defoliation and to a lesser extent by current season's shading (Table 5.5). Winkler (1934) refers to the level of carbohydrate reserves as an indication of the capacity of the vine to grow in the coming season. Linear regression analysis (Figure 5.8) illustrated that there was a close link between carbohydrate reserves (starch

concentrations) in trunks at bud burst and vine capacity at the end of the growing season. This relationship illustrates Winkler's (1934) suggestion and shows that stored CHO reserves in the trunks and roots of grapevines play an important role in overall vine growth and productivity in a cool climate environment, where the opportunity for compensatory growth is limited because of cooler temperatures and a shorter growing season. The reduction in vine capacity can be ultimately attributed to reduced flowering and shoot vigour, which in turn is most probably the consequence of reduced over-wintering CHO reserves. The relationship between reduced over-wintering CHO reserves and flowering will be discussed in 5.4.5.

Finally, it was observed that reductions in yield and pruning weight, as a consequence of previous season's defoliation and current season's shading, resulted in major shifts in the yield to pruning weight ratio (Ravaz index) (Table 5.5). Such changes indicate that reproductive growth relative to vegetative growth was more adversely affected by both defoliation and shading (Table 5.5). According to Smart *et al.* (1990) well balanced vines should have a Ravaz index between 5 and 7, yet in this experiment even non-treated vines only managed a ratio of 2kg of fruit for every 1kg of prunings (Table 5.5). Such low ratios indicate that the Chardonnay vines in this experiment were not in good balance. The low ratios may to a certain extent be a reflection of the type of Chardonnay clone (Mendoza) which has a reputation for being less fruitful than other Chardonnay clones. The low ratios may also be indicative of the possibility that vines may have been over-pruned at the end of the defoliation season (1997/1998) and consequently did not have enough nodes per vine.

5.4.4 Defoliation and shade effects in seasons two (1999/2000) and three (2000/2001)

Carbohydrate reserves as discussed in 5.4.1 had fully recovered by the start of the second season following defoliation (28/9/1999), however, data from vines shaded in the previous season revealed reasonably small but significant reductions in both root and trunk starch concentrations at bud burst (28/9/1999) compared with non-shaded vines (Table 5.6). This result supports earlier discussion that 5 weeks of shading was reducing photosynthate production. Therefore not only was previous season's shading reducing photosynthate supply to setting inflorescences, but also to the accumulation of CHO

reserves in trunks and roots. Contrary to these results, McArtney (1998) could find no effect of the previous season's shading on the concentration of starch in the roots of 2 year old pot grown Dechaunac vines at bud burst, but McArtney (1998) did find that total root mass was reduced and therefore also the total amount of root CHO reserves.

Yield components during the 1999/2000 season were still being influenced by defoliation imposed two seasons previously, but to a much lesser degree. For example, inflorescence number per shoot on 4 week defoliated vines was 17% lower than non-defoliated vines (Table 5.7), whereas in the 1998/1999 season it was 40% lower than non-defoliated vines (Table 5.1). Flower number per inflorescence was also lower in the 4 week defoliation, with approximately 15% fewer flowers than no defoliation (Table 5.7), whereas in the 1998/1999 season there was a 50% reduction in flower number (Table 5.1). In the 1999/2000 season no clear association could be found between the smaller reductions in inflorescence and flower numbers and CHO concentrations at bud burst, however a link with reduced shoot vigour was found. Linear regression analyses revealed significant relationships between shoot diameter or weight and inflorescence and flower number (data not shown). The coefficients of determination (R^2) of these relationships were relatively low at, or around, 0.50 indicating a considerable amount of variation in inflorescence and flower number was not accounted for by shoot diameter or weight. Despite this, these relationships suggest that the thinner lighter shoots (now canes) carried smaller buds that had fewer inflorescence primordia, and as consequence of this new shoots that developed from these thinner canes produced fewer inflorescences and flowers per inflorescence. Previous work by Wilson (1996) has demonstrated that smaller buds at basal node positions on a cane had fewer inflorescence primordia than larger buds at higher node positions. Therefore it is reasonable to conclude that continued reductions in vine flowering in response to 4 week vine defoliation two seasons previously were the consequence of reduced shoot vigour on bud size and the number of inflorescence primordia contained within.

Per cent fruitset during the 1999/2000 season was much lower than 1998/1999 season, and in contrast to the first season, per cent fruitset was lower in early defoliated vines compared with no defoliation (Table 5.7). The lower fruitset was not associated with CHO reserves as all defoliated vines had recovered in the previous season. However, the

overall reduction in per cent fruitset this season as well as in response to early defoliation (Table 5.7) may have been the consequence of poorer weather conditions during the 1999/2000 season. The bloom period of 1999/2000 season was dominated by cloudy cool weather which contrasted with the above average sunshine and warmth experienced in the previous 1998/1999 season (Appendix 2). The less favourable weather conditions during and after bloom were associated with a commercially unacceptable incidence of powdery mildew in the experimental vineyard, which may have preferentially affected early defoliated vines more with a considerable negative effect on the sensitive fruitset process. Powdery mildew infection has been shown to reduce the rate of leaf photosynthesis (Clearwater *et al.* 2000). Based on these factors one may conclude that reduced photosynthesis due to unfavourable weather and disease was responsible for low fruitset and that this was accentuated in early defoliated vines by a lingering defoliation stress. As a consequence of fewer flowers per inflorescence and lower per cent fruitset in early defoliated vines, berries per cluster and cluster weight were lower (Table 5.7). Lower cluster weight and fewer clusters per vine (as a consequence of reduced inflorescences per shoot) were cumulative in reducing vine yield. In fact yield was more than halved by 4 week defoliation compared with no defoliation, even though inflorescence and flower number were only reduced by 17% and 15% respectively (Table 5.7).

The effect of previous season's shading on inflorescence and flower numbers was not as severe as the effects of previous season's defoliation, for example shading reduced inflorescence and flower numbers by 20% and 12% respectively compared with no shade (Table 5.8), whereas defoliation reduced inflorescence and flower numbers by 40% and 50% respectively compared with no defoliation (Table 5.1). The less severe effect of shading on floral components is consistent with the less severe effect of shading on CHO reserves (Table 5.6). Other shading studies have also reported reduced flowering as a consequence of previous season's shading, but have fallen short of establishing a physiological cause for the reduction in flowering. For example, Ferree *et al.* (2001) showed that increasing intensities of shade in the previous season (pot grown grapevines) resulted in linear decreases in inflorescence number per shoot. Similar effects have also been observed by Keller and Koblet (1995b) with pot grown Müller Thurgau grapevines, by May and Antcliff (1963) with Sultana vines and also by Buttrose (1970). Unlike these

studies the results of the shading experiment presented in this chapter suggest that reductions in over-wintering CHO reserves may be the physiological cause of reduced flowering (Figure 5.9). The physiological relationship between CHO reserves and flowering is discussed in 5.4.5. Like the defoliation experiment, fewer flowers per inflorescence was the primary cause of fewer berries per cluster, although a tendency for reduced fruitset in shaded vines (Table 5.8) may have also contributed to the reduction in berries per cluster. Fewer berries per cluster resulted in lighter clusters, and with fewer clusters per vine (as a consequence of fewer inflorescences per shoot), the cumulative effect on yield was evident with a 40% reduction in vine yield (Table 5.8).

Carry-over effects into the third season following defoliation and into second season following shading (2000/2001) were minimal. There was, however, at the start of the season (Bud burst, 27/9/2000) a small but significant increase in the trunk starch concentrations of shaded vines (Table 5.9). This was probably related to the lower vine yields in the previous season (Table 5.8), where a lighter crop load may have meant that more photosynthates were partitioned to CHO reserves in the trunk. In the defoliation experiment large differences in previous season's crop load (Table 5.2) did not impact on CHO reserves at the start of the following season (Figure 5.3). Observations such as this cast doubt on the above explanation for an increase of CHO reserves in shaded vines. However this explanation may be valid given that the 1999/2000 season was considerably cloudier and cooler than the 1998/1990 season (Appendix 2) and the fact that powdery mildew infections had occurred. The small increases in trunk starch concentrations in shaded vines did not appear to be linked to either inflorescence or flower numbers in the 2000/2001 season. Defoliation three seasons previously had no effect on inflorescence or flower numbers. Likewise, fruitset, berries per cluster, cluster weight and yields were all unaffected (Table 5.10 and 5.11).

5.4.5 The relationship between carbohydrate reserves and floral components of yield

Previous season's defoliation has been shown by Candolfi-Vasconcelos and Koblet (1990) to reduce CHO reserves in trunks of mature grapevines. Results from the defoliation and shading experiments presented in this chapter have also illustrated this, but unlike previous studies the results presented here have demonstrated that root CHO

reserves are also reduced, and are in fact more sensitive to defoliation than trunks. Previous studies, like those of Candolfi-Vasconcelos and Koblet (1990), have not attempted to fully integrate the changes in CHO reserves with subsequent reproductive and vegetative growth and thus vine productivity. Regression analysis has revealed strong positive relationships between starch concentrations at bud burst in both trunks (Figures 5.6 and 5.9) and roots (data not shown) and the number of inflorescences and flowers formed. In vines defoliated in the previous season the logn relationship indicated that initial increases in starch concentrations resulted in large increases in inflorescence numbers, but this plateaued at higher starch concentrations (Figure 5.6a). Flowers per inflorescence however, increased in a linear fashion in response to increasing starch concentrations (Figure 5.6b). Therefore, it is probable an upper limit to this relationship would be reached where starch reserves cannot accumulate beyond a certain physiological concentration and flowers per inflorescence would become limited by other factors.

Reductions in trunk starch concentrations due to previous season's shading (Table 5.6) were less severe than defoliation treatments (Figure 5.3). Within the higher and hence tighter CHO reserve range for shaded vines, inflorescence number related to starch concentration in a linear manner (Figure 5.9a) rather than the logn curve. This was primarily because lower starch levels, where inflorescence number decreases rapidly (Figure 5.6a), were not attained by shading. The absolute values between the defoliation and shading experiments agree well at the high end of the relationships, for example defoliation with 9.5% starch yields 2.0 inflorescences per shoot, and shade with 9.5% starch also yields 2.0 inflorescences per shoot (Figures 5.6a and 5.9a respectively). However, at the lower end of the relationships, defoliation with 7% starch yields 1.8 inflorescences per shoot, while shade with 7% starch yields 1.5 inflorescences per shoot (Figures 5.6a and 5.9a respectively). This inconsistency at the lower end of the relationships is probably the result of a seasonal effect, that is, in different seasons the slope of the relationship between starch concentrations and inflorescence number varies. Despite this the relationships provide strong evidence that regardless of how CHO reserves may be reduced there is consistent negative effect on subsequent inflorescence and flower numbers.

To further study the relationship between CHO reserves and inflorescence and flower numbers, data from vines that were not defoliated or shaded over the three growing seasons were used in regressions of trunk CHO concentrations against inflorescence and flower numbers (Figure 5.10). This was done to examine whether variations in trunk CHO reserves across seasons were linked to the changes in inflorescence and flower numbers (Tables 5.2, 5.7 and 5.11) in the absence of imposed conditions, namely defoliation and shading.

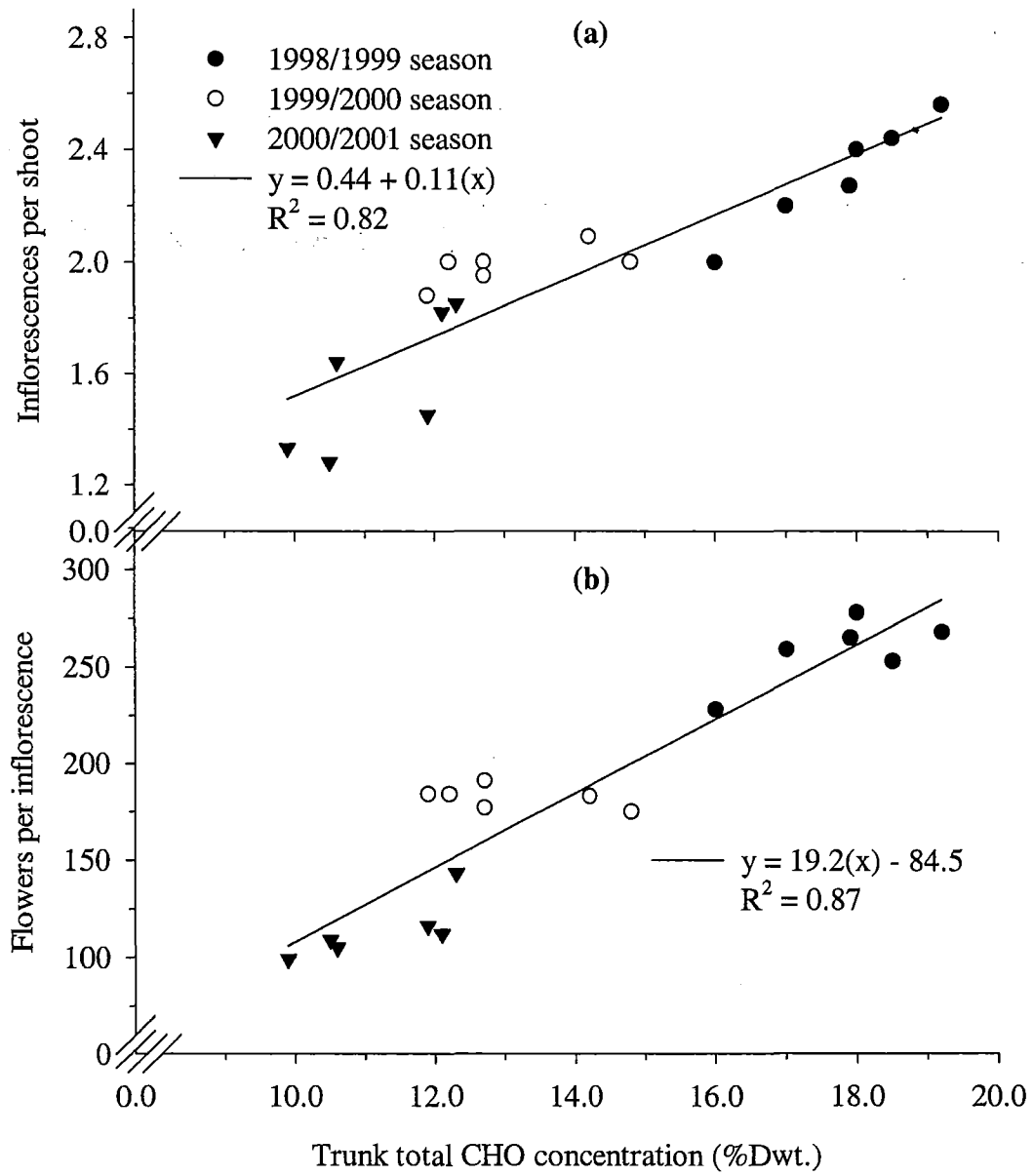

Figure 5.10 The relationship between trunk total CHO concentrations at bud burst and (a) inflorescences per shoot and (b) flowers per inflorescence over three growing seasons (1998-2001).

Of the three CHO forms, total CHO concentration was found to have the strongest positive relationship with inflorescence and flower numbers. Starch concentration on the other hand, did not show a strong relationship (R^2 = 0.45, plots not shown). This is because there were differences in starch/soluble sugar ratios in non-treated vines between each season (Figure 5.3 and Table 5.9), even though trunk sampling was always carried out at the same phenological stage (bud burst). The relationships illustrate that large differences in total CHO concentrations (e.g. from 10 to 20%Dwt) were closely associated with changes in inflorescence and flower number, but relatively small changes (e.g. 12 to 14%Dwt) were not (Figure 5.10). Data from the 1999/2000 season gave least fit to the overall relationship especially in terms of flower number (Figure 5.10b). Despite this the 1999/2000 data points did fall within the two other seasons and therefore it is reasonable to suggest that the overall relationship holds true. Given that defoliation, shading and seasonal relationships all show that CHO reserves are associated with inflorescence and flower numbers one may conclude that grapevine flowering is dependent on over-wintering CHO reserves.

The proposed link between CHO's and vine fruitfulness (inflorescence and flower number) is not new. Thomas and Barnard (1937a) suggested that a reduction of CHO supply into buds could be responsible for reduced fruitfulness. This has since been reiterated by May (1965) and Sommer *et al.* (2000), but not conclusively proven. The defoliation, shading and season data presented here have illustrated that the relationship between CHO reserves and floral components is indeed robust and therefore provides strong evidence to support the propositions of Thomas and Barnard (1937a), May (1965) and Sommer *et al.* (2000). However, the exact mechanisms involved in the relationship between CHO reserves and inflorescence number are less apparent. In terms of the sequence of floral development in the grapevine, inflorescence number per shoot is already determined within latent buds before harvest of the previous season (May 2000). Therefore it becomes evident that reductions in over-wintering trunk and root CHO cannot have a direct effect on inflorescence number, unless low CHO reserves result in the abortion of developing inflorescences in early spring. This, however, was never observed in this trial. Therefore the second most probable mechanism is that defoliation directly impacted on the initiation of inflorescence primordia, a scenario hypothesised by May (1965), Smart *et al.* (1982) and Sommer *et al.* (2000). Even a direct link between

trunk and root CHO reserves during the period of inflorescence initiation seems unlikely because the predominant flow of photosynthate from the leaves is in a downward direction towards clusters, trunks and roots at this time (Hale and Weaver 1962). Therefore the relationships between CHO reserves and inflorescence numbers do not necessary indicate cause and effect, even though they are associated.

The direct effects of defoliation on inflorescence number per shoot are supported by the fact that single node cuttings (Table 5.1) clearly indicated fewer inflorescences per shoot in defoliated vines at a time (winter) well before CHO reserves within vines were utilised in the development of new season's shoots and inflorescences. The reduced initiation of inflorescences in response to defoliation may, however, be linked to CHO supply. Results presented here clearly show that defoliation and shading, but possibly also disease and unfavourable weather, reduced the amount of photosynthate for CHO reserve accumulation (Figures 5.2 and 5.3, Table 5.6) during the initiation period. Likewise reduced photosynthate supply was implicated as the cause of reduced fruitset (Table 5.2). Therefore it is not unreasonable to suggest that photosynthate supply to latent buds during the period of inflorescence initiation (which occurs at the same time as CHO reserve accumulation and fruitset i.e. bloom to pre-véraison) was also reduced with the result of significantly impeding inflorescence initiation activity.

The relationship between over-wintering CHO reserves and flower number per inflorescence (Figure 5.6b) must be considered differently to inflorescence number per shoot because flower number per inflorescence is not finally determined until the period during and after bud burst (May 1964, 2000). Results presented in this chapter clearly revealed major reductions in CHO reserves at exactly the same time (bud burst) as flower number was being determined (Figures 5.2 and 5.3, Table 5.6). Therefore the fact that fewer flowers per inflorescence were measured on vines with reduced CHO reserves suggests CHO reserves may have been directly involved in the determination of flower number per inflorescence, a proposition first suggested by Scholefield *et al.* (1977). Scholefield *et al.* (1977) speculated that a reduction in flowers per inflorescence (in response to previous season's pruning at harvest) was a consequence of reduction in CHO reserves where harvest pruning had prematurely killed off 60% of a grapevine's leaf area. The direct involvement of CHO reserves is further supported by literature

which shows that inflorescence primordia undergo secondary and tertiary branching, and then finally flower formation at bud burst (Barnard and Thomas 1933, Carolus 1970, May 2000). It is therefore quite conceivable that reductions in flower numbers were the consequence of reduced secondary and tertiary branching and flower formation where CHO reserves were limiting.

5.5 Conclusions

The results of the experiments discussed in this chapter have shown that the concentration of CHO's reserves in the trunks and roots of grapevines can vary from season to season. Treatments such as defoliation and shading have also been shown to have negative effects on the concentrations of CHO reserves in both trunks and roots which in turn were correlated with following season's reproductive and vegetative growth, in particular inflorescence and flower numbers and shoot vigour. Depending on the extremity of such events recovery in floral components (inflorescence and flower numbers) took one or two seasons. Second season effects were associated with less vigorous shoots (canes). Carbohydrate reserves usually recovered within one season.

The findings of the experiments presented in this chapter illustrate that there is now good reason to believe that CHO reserves during the bud burst period play an important role in the development of the inflorescence, that is, final branching and determination of flower number. Per cent fruitset was not directly affected by reductions in CHO reserves, but as previous research has shown was highly dependent on photosynthate supply during the bloom period. Results indicate CHO reserves are not involved in the initiation of inflorescences because of the difference in time between inflorescence initiation and the utilisation of CHO reserves in new season's shoot and inflorescence development. Despite this, results indicate the two may always be associated because of their links to photosynthates sourced from leaves. One may speculate that when photosynthate supply throughout the grapevine is reduced as a result of an extreme event (defoliation, shading, disease or unfavourable weather), the inflorescence initiation process in latent buds may be deprived of CHO's and hence inflorescence number per shoot in the following season is reduced. Such speculation warranted further investigation and is documented in the experiments described in Chapter 6.

Chapter 6

The effect of shoot leaf removal and vine defoliation on inflorescence initiation

6.1 Introduction

Results discussed in Chapter 5 demonstrated that the concentration of over-wintering CHO reserves in the trunks and roots of mature grapevines was associated with the number of inflorescences formed per shoot and the number of flowers formed per inflorescence in the following spring. However, discussion suggested that the association between CHO reserves and inflorescence number per shoot was not directly cause and effect because inflorescences were initiated in the season previous to fruiting (May 2000). Therefore it is probable that the number of inflorescences per shoot was set before over-wintering CHO reserves were utilised in new season's shoot and inflorescence development. Measurement of bud fertility using single node cuttings confirmed that inflorescence number was set in the season previous to fruiting. Thus it is probable that the reduction in inflorescence number per shoot was the result of the effects of defoliation on inflorescence initiation. The physiological cause for the reduction in inflorescence initiation in response to defoliation was not identified in Chapter 5, however previous literature (May 1965, Sommer *et al.* 2000, Thomas and Barnard 1937a) suggests that the supply of carbohydrates may be integral to the successful initiation of inflorescences in latent buds. Based on this literature and the findings of Chapter 5 the primary focus of the experiment described in this chapter was to establish

that defoliation reduces inflorescence number per shoot and that a reduction in carbohydrate supply, where photosynthate source leaves have been removed, is the physiological cause of reduced inflorescence initiation.

In order to investigate this focus an individual shoot leaf removal and vine defoliation experiment was carried out. The first aim of the experiment was to examine how shoot leaf removal (to reduce CHO supply) at different times during the growing season affected the initiation of inflorescences in developing latent buds, and how the accumulation of CHO's in shoot tissue may be related to this. The second aim of the experiment was to examine if vine defoliation would interact with the individual shoot leaf removal in terms of inflorescence initiation. This interaction effect was included to determine whether or not defoliation of other parts of the vine could affect the initiation of inflorescences on selected shoots at certain times during the growing season.

Vine defoliation treatments also provided the opportunity to examine the effects of defoliation on over-wintering trunk and root CHO reserves, sap flow at bud burst and subsequent flowering, but under the influence of different numbers of nodes retained per vine after winter pruning. Retention of more nodes per vine has been found to reduce bud fertility in the following season (Clingeleffer 1984), however a mechanism and physiological cause for the reduction in bud fertility has not been determined. One may speculate that the abortion of initiated inflorescences shortly after bud burst is the mechanism for the reduction in bud fertility and that inadequate CHO supply may be the physiological cause. Measurements of sap flow at bud burst in the season following vine defoliation were carried out to determine whether or not changes in the concentrations of over-wintering trunk and root CHO reserves would affect sap flow rates and whether this in turn had any association with subsequent vine flowering and productivity. Previous work by McArtney (1998) and Togonidze (1985) suggests that the size of the root system does influence sap flow rates at bud burst. Therefore one possible advantage to measuring sap flow is that it may integrate both root system size and the concentrations of CHO reserves within and therefore provide a more accurate assessment of the mobilisation and the availability of reserve CHO's following bud burst. If this is so, sap flow may show even stronger relationships with subsequent grapevine flowering and productivity than trunk and root CHO concentrations.

6.2 Materials and Methods

6.2.1 Shoot leaf removal and vine defoliation

Mature VSP cane trained (two canes, ten nodes each) Chardonnay vines (own roots, clone unknown) growing in the Lincoln University vineyard were used for the shoot leaf removal and vine defoliation experiment, which commenced in early summer 1998. Nine shoots (selected on the basis of similar size and vigour) per vine were randomly assigned a time of leaf removal treatment. Leaf removal treatments consisted of 100% leaf removal of each shoot (Figure 6.1) with the first treatment imposed at 50% bloom (approximately the beginning of inflorescence initiation, May (2000) and there after at 2 weekly intervals up to 14 weeks post-bloom. Fifty per cent bloom occurred on Dec 15 1998, hence subsequent treatment shoots had their leaves removed on the following dates: Dec 29 (2 weeks), Jan 12 (4 weeks), Jan 26, (6 weeks), Feb 9 (8 weeks), Feb 23 (10 weeks), Mar 9 (12 weeks), and Mar 23 (14 weeks). The ninth treatment shoot had no leaves removed (natural leaf fall at 19 weeks post-bloom). Fruit clusters (usually two per shoot, borne on nodes three to five) were not removed and no shoot topping was performed, therefore as new leaves developed they were removed according to the individual shoot leaf removal treatment.

In addition to individual shoot leaf removal the remaining shoots on each vine were fully defoliated on either Dec 15 (0 weeks), Feb 9 (8 weeks) or not at all (no defoliation, natural leaf fall 19 weeks post-bloom). The experiment was set up as a three x nine randomised split plot design with main plots represented by individual vines defoliated at 0 or 8 weeks post-bloom or not at all and subplots were represented by the nine individual leaf removal treatments. The vine defoliation and the nine individual shoot leaf removal treatments were replicated 12 times (36 vines were used). Although not the focus of this study basic measurements of fruit weight and juice composition and pruning weight were recorded from the vines at the end of the defoliation season (Appendix 3).

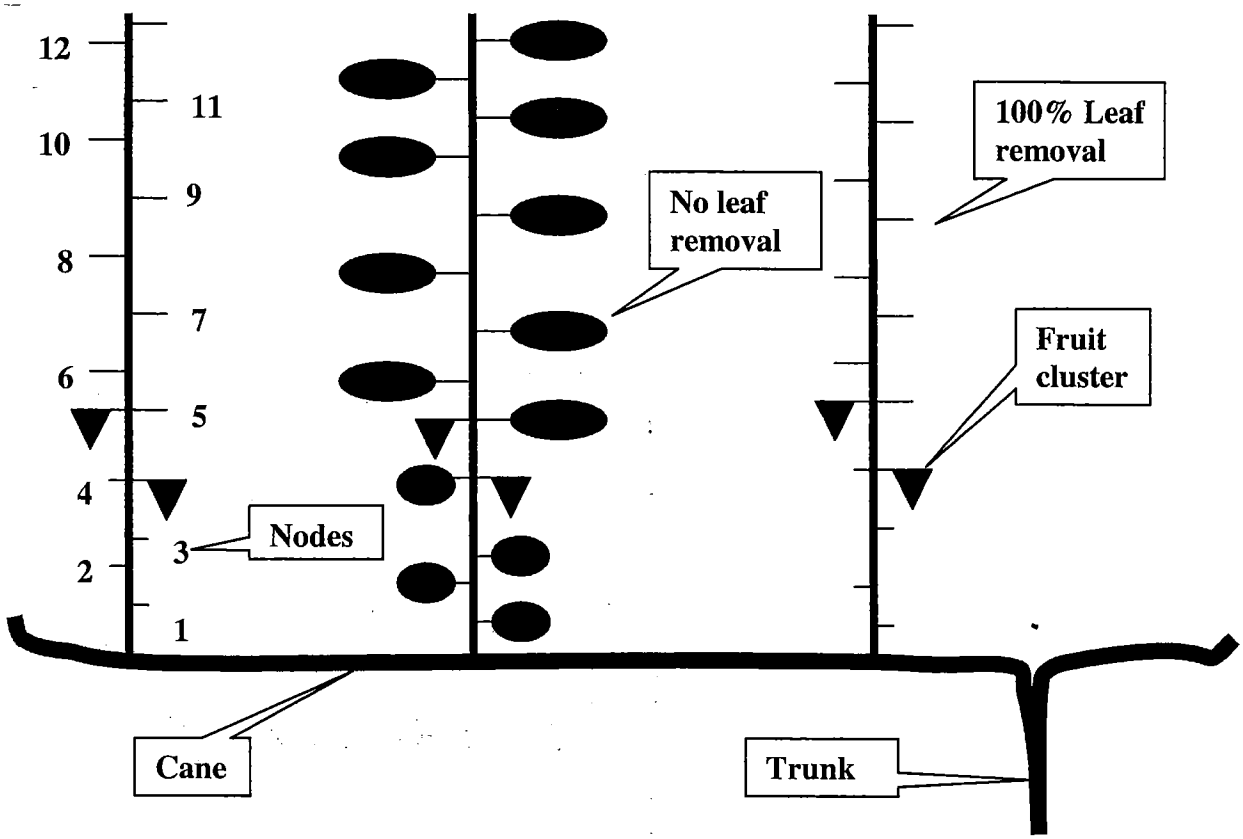

Figure 6.1 Diagrammatic representation of individual shoot leaf removal treatments. Numbers indicate the node positions for which inflorescence initiation was examined (see 6.2.2 for details).

6.2.2 Shoot carbohydrate sampling and inflorescence initiation

The twelve replicates of the experiment were divided into two groups of six replicates. The first group of replicates were used for both shoot CHO sampling throughout the growing season and inflorescence initiation examination. The accumulation of shoot CHO's as the season progressed was studied at three leaf removal timings, that is, Dec 15 (0 weeks), Feb 9 (8 weeks) and no leaf removal (natural leaf fall). Four sample times were chosen to study the accumulation of shoot CHO's at the sixth internode, these were pre-véraison, véraison, mid-ripening and leaf fall, 31, 63, 92 and 151 days after 50% bloom respectively. A fifth sample was taken just before bud burst (24/9/1999), 276 days after 50% bloom. Sample shoots were chosen at random, but avoiding those that arose from spurs set aside for next season's canes. Shoots were cut off above the 5th node and immediately frozen for CHO analysis in the laboratory. The portion of the sample shoot (node 1 to 5) still attached to the vine was treated according to leaf removal

treatment. The first three shoot samples were removed before harvest and the fourth sample taken at leaf fall. Although partial shoot removal would have reduced vine leaf area, it was considered that with only three shoots partially removed before leaf fall that this would have had minimal effect on the vine as a whole and therefore would not distinguish this group of replicates from the second group of replicates.

The nine individual shoot leaf removal treatments (referred to as canes post-leaf fall) from the first group of six replicates were selected for inflorescence initiation examination. The single node cutting technique was used to measure the number of inflorescences initiated per node. This involved removing the nine treatment canes from the vines in early winter 1999 and cutting the first twelve node positions per cane (Figure 6.1) into single node cuttings. The cuttings were placed in water and left to grow in a heated glasshouse until the inflorescences could be easily counted. Shortly after the single node cuttings were made in Winter 1999 the second group of six replicates were pruned back to the nine individual treatment canes plus spur cane(s). The nine individual treatment canes were tipped to twelve nodes and trained horizontally and left to grow (see Plates 6.1, 6.2) to examine whether or not the effects of leaf removal on inflorescence initiation could also be observed on the vine (field validation of the single node cuttings). Both cane bud burst and inflorescence number per shoot for each of the 12 nodes was measured in the following growing season. The first group of six replicates from which both shoot CHO samples and single node cuttings had been taken were pruned back to standard cane VSP (two canes with ten nodes on each and two spurs).

6.2.3 Sap collection and trunk and root carbohydrate sampling

Bleeding sap volume over a 24 hour period was measured at the start of the season following defoliation (bud burst, 22-23/9/1999). To collect the sap one spur cane per vine was cut at the 10^{th} internode and connected to rubber tubing, which led to a collection container placed on the ground beside the vine (Plate 6.3). To avoid soil and rain contamination and evaporation the container was sealed inside a plastic bag. A small hole in the container lid and bag was made to allow for air displacement (Plate 6.4).

Plate 6.1 Leaf removal shoots from the previous season laid down as canes to grow in the coming season.

Plate 6.2 New shoot growth developing from the canes that had their leaves removed in the previous season. Note the inflorescences developing on the new shoots (white circle).

Plate 6.3 Spur canes connected to rubber tubing and collection containers.

Plate 6.4 Sap collection. Note the sap in the bottom of the container.

The concentration of soluble sugar in the collected sap was measured using the Anthrone colorimetic test described in 3.1. Sap sub-samples had to be first diluted by a factor of ten to fall within the Anthrone standard range. Trunk (5mm core) and root (1 – 1.5cm diameter) CHO samples were taken immediately after sap collection (24/9/1999) and analysed using the methods described in Chapters 3 and 5 (see 3.1 and 5.2.2). In addition to sap and CHO measurements, inflorescences per shoot were counted one month after bud burst. Berries per cluster, cluster weight and cane and whole vine yields were measured at harvest. Shoot growth, in terms of diameter at the 5th internode and fresh weight, and whole vine pruning weight were measured after leaf fall.

6.2.4 Statistical analysis

All vine data from the three x nine factorial split plot experiment were analysed using general ANOVA testing for polynomial (linear and quadratic) significance using a Genstat statistical package (Genstat 5 Release 4.1. Copyright 1997, Lawes Agricultural Trust, Rothamsted Experimental Station). Quadratic significance testing was included because of the non-linear increase in time period between the last shoot leaf removal treatment at 14 weeks post-bloom and no leaf removal (natural leaf fall at 19 weeks post-bloom), that is, 5 week time period versus 2 weeks for leaf removal treatments previous to 14 weeks post-bloom. Mean separations were determined utilising least significant difference (lsd) at the 5% level of significance. Simple and multiple linear and non-linear regressions of scatter plots were performed using Genstat and plotted using Sigma plot (Sigma plot for Windows version 4.01. Copyright 1986-1997 SPSS Inc.).

6.3 Results

Analysis of data using ANOVA indicated that there were no significant interactions between the individual shoot leaf removal and vine defoliation in terms of any reproductive or vegetative growth from individual shoots. Consequently data for individual shoot leaf removal treatments and whole vine defoliations are presented separately.

6.3.1 Individual shoot leaf removal

6.3.1.1 Shoot carbohydrate accumulation

Pre-véraison (31 days after the first leaf removal treatment) shoot soluble sugar concentrations ranged between 7.8-9.0% on dry weight basis with no difference between defoliations (Figure 6.2a). Further measurements at véraison, mid-ripening and leaf fall (63, 92 and 151 days after first leaf removal treatment respectively) also revealed no difference between leaf removal treatments, however at bud burst (276 days) 8 week leaf removal had higher sugar concentrations than no leaf removal (Figure 6.2a). Shoot starch concentrations 31 days after bloom were less than 1% on dry weight basis and no leaf removal effect could be detected (Figure 6.2b). At 63 days 0 week shoots had 2%Dwt starch while 8 week and no leaf removal shoots had 6%Dwt starch. At 92 days starch concentrations in 0 week shoots had increased to 3%Dwt, but no increase was observed in 8 week shoots, while no leaf removal shoot starch concentrations had increased to 11%Dwt. Just after leaf fall (151 days) 0 week shoot starch concentrations had climbed to 5%Dwt, while no further increase in starch concentrations occurred in the 8 week and no leaf removal shoots. Starch concentrations at bud burst remained the same as those at leaf fall (Figure 6.2b).

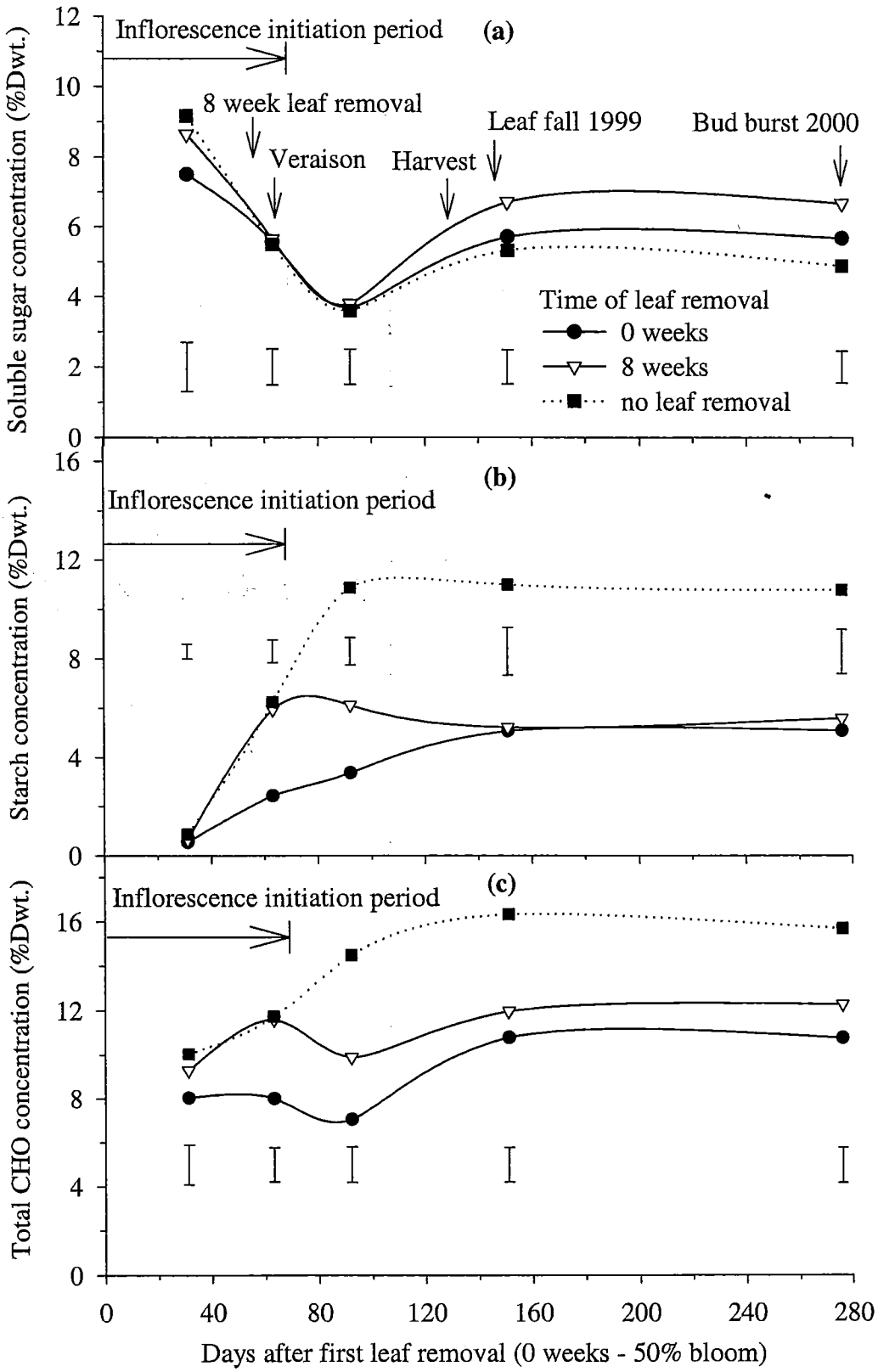

Figure 6.2 The effect of leaf removal at 0 and 8 weeks post-bloom and no leaf removal on the accumulation of (a) soluble sugar, (b) starch and (c) total CHO at the 6th internode of shoots.
Bars represent lsd at 5% level of significance.

6.3.1.2 Bud burst and inflorescence number

Per cent bud burst, for canes still attached to the vine, was lower the earlier leaves were removed in the previous season (Table 6.1). For example, leaf removal at bloom (0 weeks) reduced bud burst to 43% (5.2 shoots) compared with no leaf removal which had 87% bud burst (10.1 shoots). Inflorescence number per shoot was not altered by time of leaf removal (Table 6.1). However, the reduction in inflorescence number per cane was the result of fewer shoots, which in turn was the consequence of reduced bud burst (Table 6.1).

Almost all the single node cuttings developed shoots and hence bud burst was high, between 90-100% (data not shown). Despite the high bud burst the number of inflorescences per shoot was reduced by previous season's leaf removal (Table 6.1). For example, leaf removal at bloom (0 weeks) reduced inflorescences per shoot to 0.29 versus 1.55 inflorescences per shoot for no leaf removal (Table 6.1). In contrast to vine measurements, the reduction in the number of inflorescences per cane was the result of reductions in inflorescences per shoot (Table 6.1).

The decrease in inflorescence number per shoot in response to previous season's leaf removal, shown by the single node cuttings, was further examined in relation to each of the twelve node positions per cane. Significant changes in inflorescence number per shoot between leaf removal and no leaf removal at each of the twelve node positions were evident (Figure 6.3). For example, inflorescence number per shoot for the earliest leaf removal treatment (0 weeks) was lower than no leaf removal from node position two onwards (Figure 6.3). For 2 and 4 week leaf removal treatments the reduction occurred from node four and five onwards respectively, at 6 weeks the reduction occurred from node seven onwards and by 10 weeks there was no difference from no leaf removal at any node position (Figure 6.3). Assessment of the node position effect on the vine was not possible because of significant reductions in bud burst (see Table 6.1). With less than 50% bud burst for the earliest leaf removal there were insufficient data to perform statistical analysis of leaf removal by node position effects.

Table 6.1 The effect of time of leaf removal in the previous season on per cent bud burst, shoot number, inflorescence number per shoot and per cane for vine measurements and inflorescence number per shoot and per cane for single node measurements.

Leaf removal (weeks post-bloom)	0 weeks	2 weeks	4 weeks	6 weeks	8 weeks	10 weeks	12 weeks	14 weeks	No Leaf R.	[1]Linear Sig.	[1]Quad. Sig.
Per cent bud burst per cane (Vine)	43.1 a[2]	54.1 b	60.7 b	76.4 cd	71.8 cd	77.2 d	83.3 de	84.3 de	87.0 e	***	NS
Shoot number per cane (Vine)	5.2 a	6.5 b	7.3 b	9.2 cd	8.6 c	9.3 cd	10.0 d	10.1 d	10.4 d	***	NS
Inflorescence number per shoot (Vine)	0.96	1.01	0.95	0.82	1.04	1.00	0.95	1.08	1.17	NS	NS
Inflorescence number per cane (Vine)	4.8 a	6.5 ab	6.9 b	7.5 bc	8.9 c	9.3 c	9.6 c	11.0 cd	12.1 d	***	NS
Inflorescence number per shoot (Single node)	0.29 a	0.47 a	0.67 b	1.06 c	1.17 c	1.36 cd	1.30 cd	1.25 c	1.55 d	***	NS
Inflorescence number per cane (Single node)	3.4 a	5.7 b	7.4 b	12.3 c	13.8 c	16.2 d	15.6 cd	14.9cd	18.5 e	***	**

[1]Linear and quadratic significance at $P \leq 0.001$ (***), $P \leq 0.01$ (**) or not significant (NS). [2]Means within the same row with the same letter are not significantly different at $P \leq 0.05$.

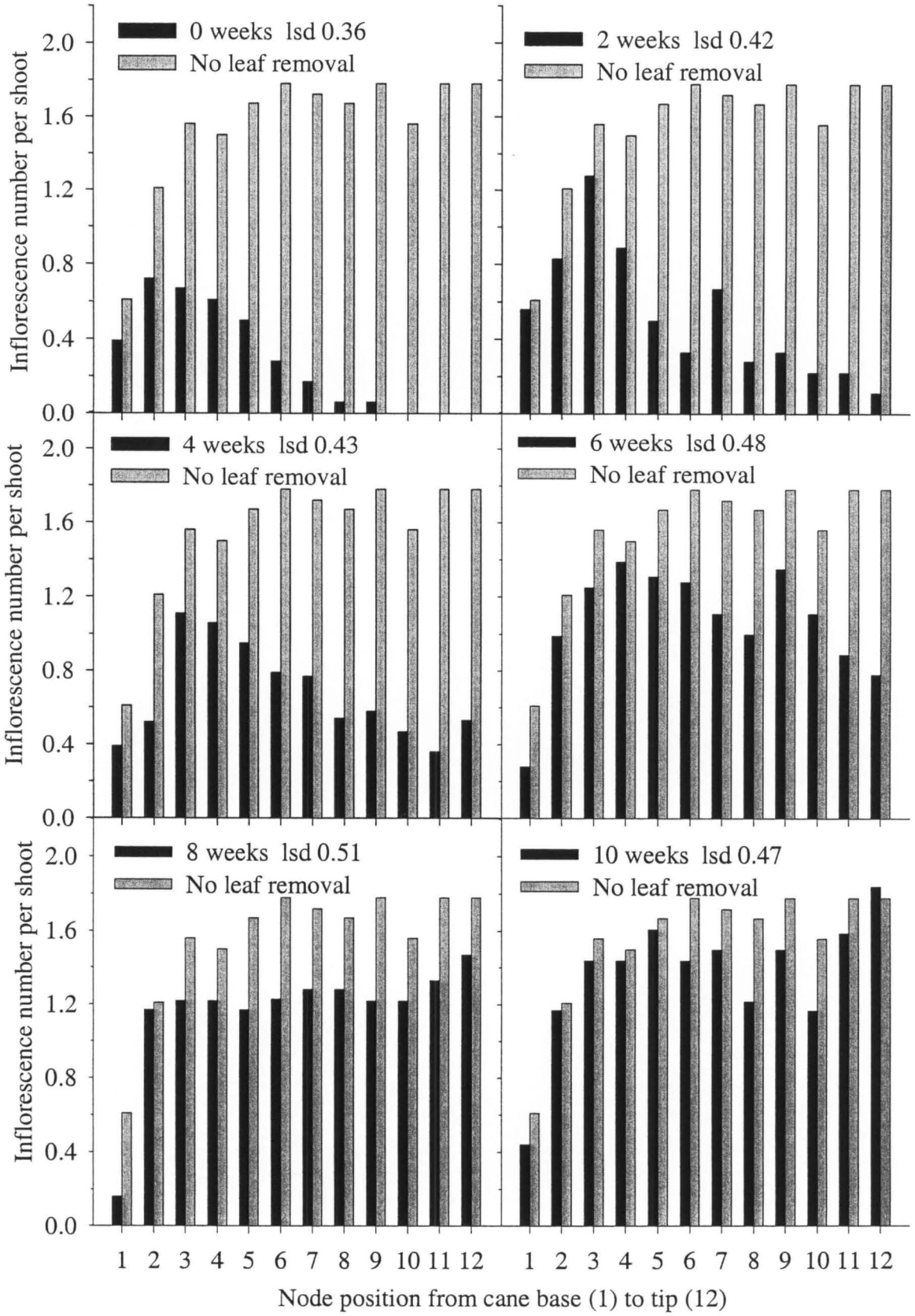

Figure 6.3 The effect of cane node position and leaf removal from 0 to 10 weeks post-bloom in the previous season on the number of inflorescences per shoot (Single node cuttings). lsd's at 5% level of significance.

6.3.1.3 Inflorescence number, bud burst, and shoot starch relationships

Because inflorescence number, bud burst and shoot CHO data for each leaf removal treatment came from different shoots (different replicate groups) direct regression analysis across replications was not possible. Therefore to integrate previous season's shoot CHO data with inflorescence and bud burst data averages for each treatment were regressed.

Both inflorescences per shoot and per cane (from single node cuttings) were found to be associated with shoot starch concentration shortly after the end of the inflorescence initiation period in the previous season (92 days post-bloom) (Figure 6.4). Inflorescences per shoot and per cane responded to shoot starch concentration in a quadratic manner. A doubling in starch concentration from approximately 3%Dwt to 6%Dwt was associated with a significant increase in both inflorescences per shoot and per cane, but further increases in starch concentration resulted in smaller increases in inflorescence per shoot and per cane (Figure 6.4).

Regression analysis indicated there was a significant ($P \leq 0.001$) quadratic relationship between shoot starch concentration at 92 days after bloom in the previous season and per cent bud burst (Figure 6.5). The quadratic nature of the relationship illustrated that initial increases in starch concentration were associated with large increases in per cent bud burst, but at higher starch concentrations (8%Dwt and above) there was little or no increase in per cent bud burst. However, no relationship between shoot starch concentrations at leaf fall (151 days) and at bud burst (276 days) and per cent bud burst could be found (Figure 6.5).

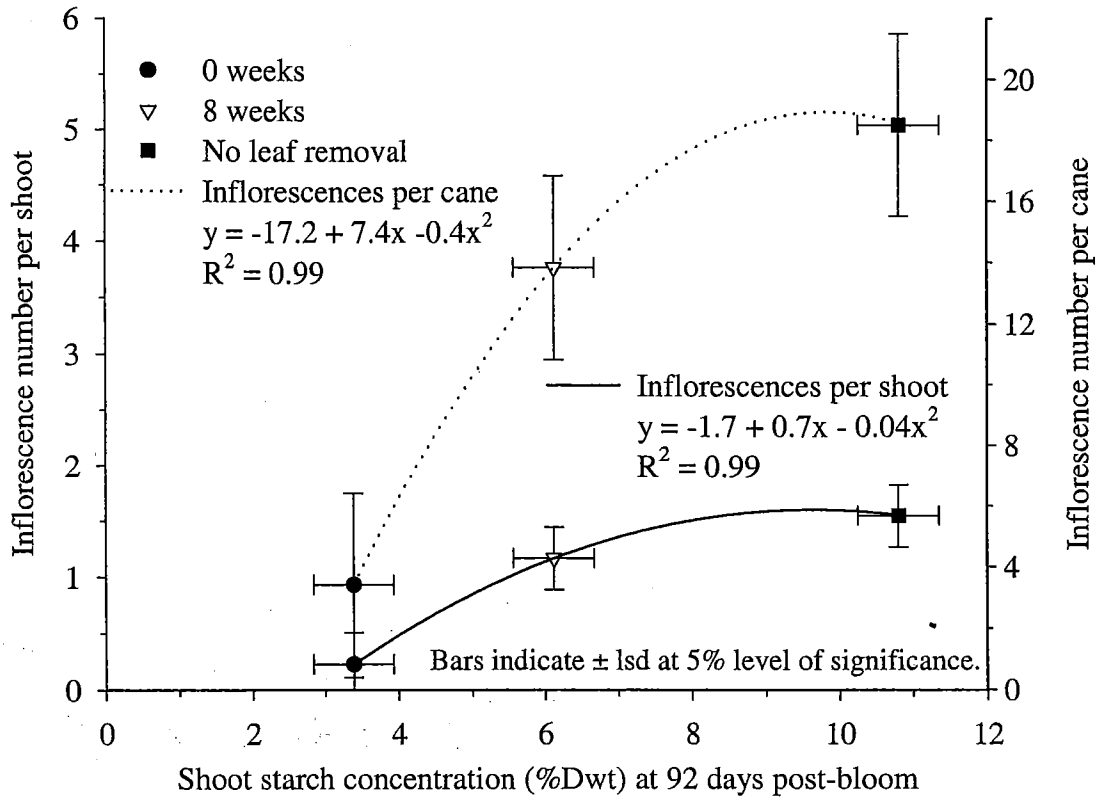

Figure 6.4 The relationship between shoot starch concentration at 92 days post-bloom and inflorescences per shoot and per cane in the following season (Single node cuttings).

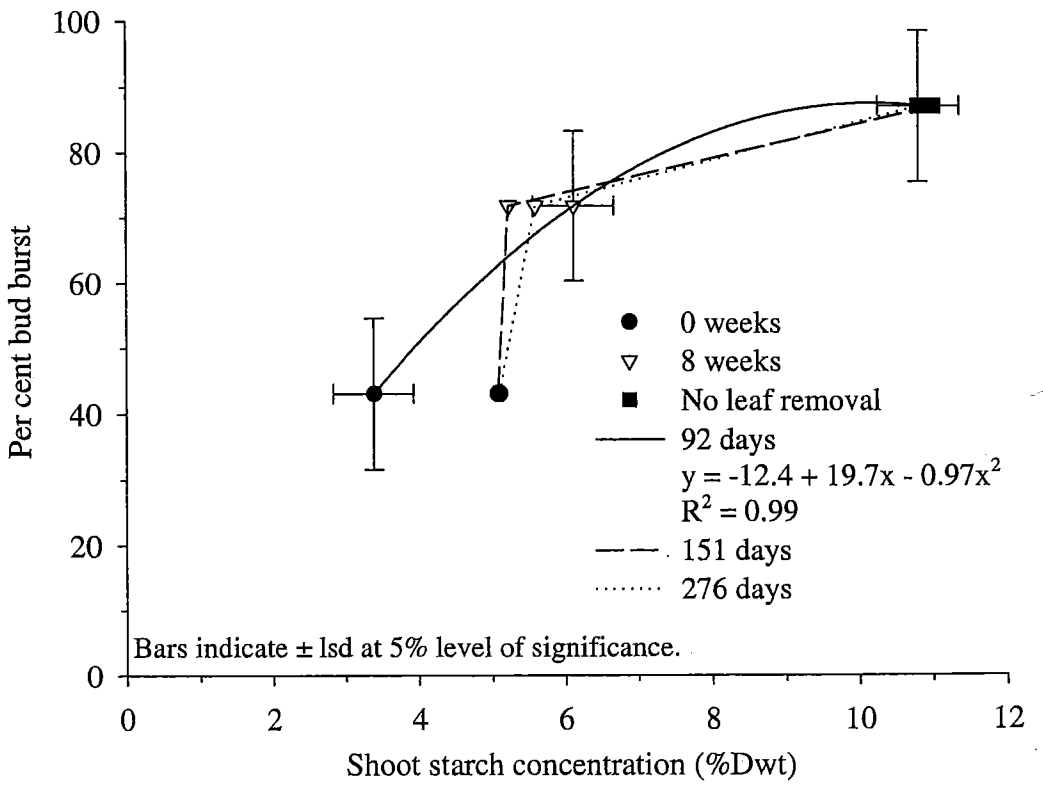

Figure 6.5 The relationship between shoot starch concentration at 92, 151 and 276 days post-bloom and per cent bud burst at the start of the following growing season (24/9/1999).

6.3.1.4 Cluster size and cane yields

Assessment of cluster size was done on every second leaf removal treatment, hence data are presented on a 4 weekly basis. The number of berries per cluster, mean berry weight and cluster weight were all unaffected by the time of previous season's leaf removal (Table 6.2). In contrast to cluster size, the number of clusters per cane was reduced, the earlier leaf removal was performed in the previous season. This was a direct result of the reduction in inflorescences per cane (Table 6.1). As a consequence of reduction in clusters per cane, cane yields were also reduced. Yields from earliest leaf removal were less than 50% of no leaf removal (Table 6.2).

Table 6.2 The effect of time of individual cane leaf removal (weeks post-bloom) in the previous season on berries per cluster, mean berry weight, cluster weight and cane yields.

Cane leaf removal	0 weeks	4 weeks	8 weeks	12 weeks	No Leaf R.	[1]Linear Sig.	[1]Quad. Sig.
Berries per cluster	43 a[2]	41 a	42 a	43 a	46 a	NS	NS
Mean berry weight (g)	0.75 a	0.71 a	0.73 a	0.75 a	0.72 a	NS	NS
Cluster weight (g)	33.9 a	30.9 a	31.9 a	33.9 a	34.9 a	NS	NS
Clusters per cane	5.4 a	7.0 b	8.5 b	9.4 bc	11.5 c	***	NS
Yield per cane (g)	167 a	213 ab	281 bc	323 cd	408 d	***	NS

[1]Linear and quadratic significance at $P \leq 0.001$ (***) or not significant (NS). [2]Means within the same row with the same letter are not significantly different at $P \leq 0.05$.

Simple linear regression indicated that inflorescences per cane and clusters per cane accounted for the majority of the variation in cane yields (R^2 = 0.84 and 0.79 respectively, $P \leq 0.001$). Multiple linear regression indicated that even more variation in cane yield was accounted for by clusters per cane + berries per cluster (R^2 = 0.92, $P \leq$ 0.001), and that nearly all the variation in cane yield was accounted for by clusters per cane + berries per cluster + berry weight (R^2 0.96, $P \leq 0.001$).

6.3.2 Vine defoliation and node number

As a consequence of the use of the nine leaf removal canes from the first group of replicates for single node cuttings, individual vines were left with approximately 20 nodes. To standardise node number per vine (to exactly 20) for the first group of replicates, vines were pruned to standard VSP (two 10 node canes - 20 nodes per vine). The second group of replicates (where the nine 12 node leaf removal canes were retained on the vine) were left with 108 nodes per vine. Therefore as a consequence of different node number per vine, comparison of vine defoliation effects were further divided into vines that had either 20 or 108 nodes.

6.3.2.1 Carbohydrate reserves and sap flow

Carbohydrate and sap measurements taken at bud burst (24/9/1999) revealed that the previous season's vine defoliation had marked effects on the concentration of carbohydrates in the trunks and roots and the amount of sap exuded (Table 6.3). Starch concentrations in the roots and trunks of early (0 weeks) and late (8 weeks) defoliated vines were reduced to half those of no defoliation (Table 6.3). In contrast to starch, soluble sugar concentrations in the roots and trunks of both early and late defoliated vines were higher than those in non-defoliated vines (Table 6.3). Either early or late defoliation in the previous season reduced bleeding sap flow (volume) at bud burst by approximately 40mL over a 24 hour period compared with no defoliation (Table 6.3). However, the concentration of sugar in the sap was not influenced by previous season's vine defoliation (Table 6.3). Linear regression analysis illustrated that sap flow was strongly (R^2 = 0.83) associated with root starch concentrations (Figure 6.6). Node number per vine had no effect on trunk and root CHO concentrations, sap flow or sap sugar concentration (data not shown).

Table 6.3 The effect of vine defoliation at 0 and 8 weeks post-bloom in the previous season or not at all on trunk and root CHO concentrations, sap flow and sap sugar concentration at bud burst (24/9/1999).

Vine defoliation (weeks post-bloom)	0 weeks	8 weeks	No defol.	[1]Linear Sig.	[1]Quad Sig.
Trunk soluble sugars (%Dwt)	4.8 a[2]	5.4 a	3.0 b	**	*
Trunk starch (%Dwt)	6.5 a	5.6 a	10.7 b	***	***
Trunk Total CHO (%Dwt)	11.3 a	10.9 a	13.7 b	***	***
Root soluble sugars (%Dwt)	4.7 a	5.4 a	4.0 b	*	**
Root starch (%Dwt)	6.8 a	3.6 a	15.3 b	***	***
Root Total CHO (%Dwt)	11.5 a	9.0 a	19.2 b	***	***
Sap volume in 24 hours (mL)	138 a	128 a	174 b	*	*
Sap sugar concentration (mg/mL)	5.1 a	5.2 a	5.7 a	NS	NS

[1]Linear and quadratic significance at $P. \leq 0.001$ (***), ≤ 0.01 (**), ≤ 0.05 (*) or not significant (NS).
[2]Means within the same row with the same letter are not significantly different at $P \leq 0.05$.

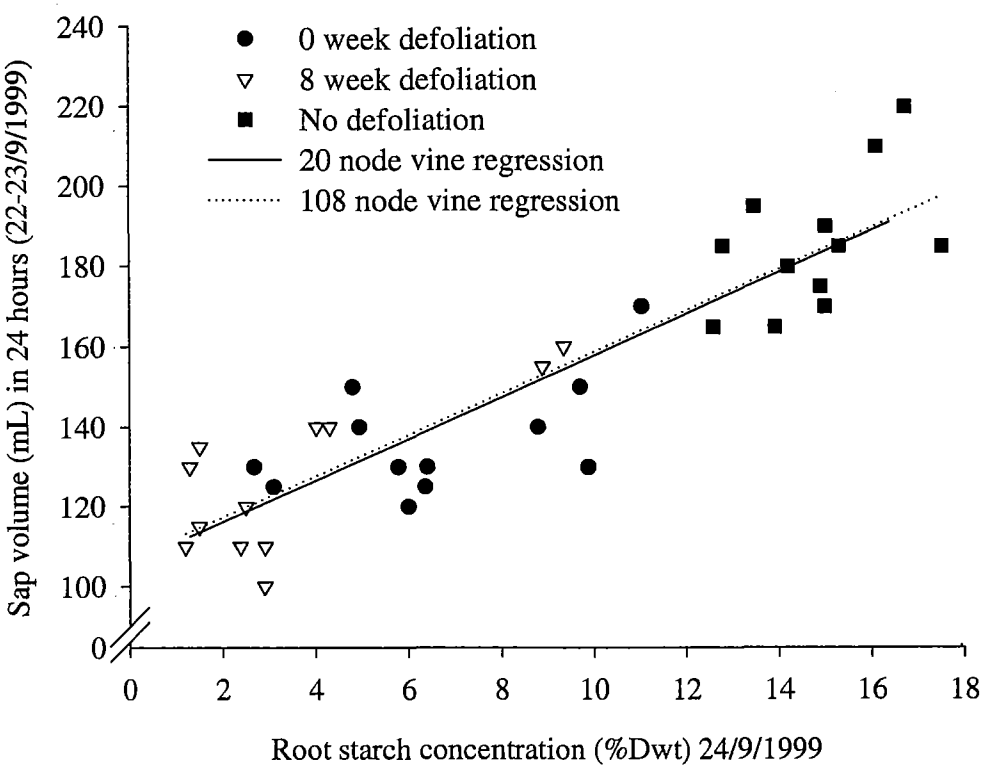

Figure 6.6 The linear relationship between root starch concentration and sap volume collected for 24 hours at bud burst (24/9/1999)
Regression for all vines $y = 105.8 + 5.2x$ $R^2 = 0.83$.

6.3.2.2 Bud burst, inflorescence number, cluster size and vine yields

Per cent bud burst was not significantly altered by previous season's defoliation. In general, shoot number was also unaffected by defoliation except for early defoliation (0 weeks) on 108 node vines where shoot number was lower than no defoliation (Table 6.4). Inflorescence number per shoot was reduced by both early (0 weeks) and late (8 weeks) defoliation on 108 node vines, but for 20 node vines, only early defoliation reduced inflorescence number per shoot compared with no defoliation. A significant interaction effect between defoliation and node number was found for inflorescence number per vine (Table 6.4). On 108 node vines, either early or late defoliation reduced inflorescence number per vine by approximately a third compared with no defoliation, but for 20 node vines either early or late defoliation did not reduce inflorescence number per vine (Table 6.4). Berry number per cluster, and berry and cluster weight were unaffected by the previous season's defoliation treatments. Cluster number per vine, like inflorescence number, was reduced by approximately a third compared with no defoliation on 108 node vines, but not reduced by defoliation on 20 node vines (Table 6.4). Vine yields were reduced by more than 1000g by both early and late defoliation on 108 node vines compared with no defoliation, on 20 node vines such an effect was not significant.

The retention of 108 nodes per vine resulted in reduced percentage bud burst compared with 20 node vines, but an overall increase in shoot number per vine resulted in more inflorescences and clusters per vine and therefore yield compared with 20 node vines (Table 6.4).

With little change in berry number per cluster and weight in response to either previous season's defoliation or node number (Table 6.4), the majority (96%) of the variation in vine yield was accounted for by inflorescence and hence cluster number per vine (Figure 6.7). The primary cause of the variation in inflorescence number per vine differed for 20 and 108 node vines. In 20 node vines, shoot number per vine accounted for more (48%) of the variation in inflorescence number per vine than inflorescence number per shoot. In 108 node vines inflorescence number shoot accounted for more (87%) of the variation in inflorescence number per vine than shoot number (Table 6.5).

Table 6.4 The effect of vine defoliation at 0 and 8 weeks post-bloom in the previous season or not at all and node number per vine on bud burst, shoot and inflorescence number per vine, cluster size and vine yields (1999/2000).

Node number per vine	**20 nodes**			**108 nodes**			**Significance**		
Vine defoliation (weeks post-bloom)	**0 weeks**	**8 weeks**	**No defol.**	**0 weeks**	**8 weeks**	**No defol.**	**[1]Nodes**	**[2]Defol**	**[3]Interaction**
Per cent bud burst	95 a[4]	90 a	100 a	65 b	70 b	76 b	**	NS	NS
Shoot number per vine	19 a	18 a	20 a	70 b	76 bc	82c	***	*	*
Inflorescence number per shoot	1.25 a	1.43 ab	1.57 b	0.98 c	0.83 c	1.28 a	***	*	NS
Inflorescence number per vine	25 a	26 a	30 a	69 b	62 b	104 c	***	***	**
Berry number per cluster	47	46	53	46	48	47	NS	NS	NS
Berry weight (g)	0.75	0.76	0.75	0.73	0.71	0.73	NS	NS	NS
Cluster weight (g)	37.5	37.9	54.2	35.7	36.4	35.5	NS	NS	NS
Cluster number per vine	23 a	27 a	28 a	70 b	62 b	104 c	***	**	**
Yield per vine (g)	810 a	950 a	1150 a	2260 b	2050 b	3370 c	***	**	*

[1], [2], & [3] main effects of Nodes, Defoliation and Interactions respectively at $P. \leq 0.05$ (*), ≤ 0.01 (**), ≤ 0.001 (***) or not significant (NS).
[4]Means within the same row with the same letter are not significantly different at $P \leq 0.05$.

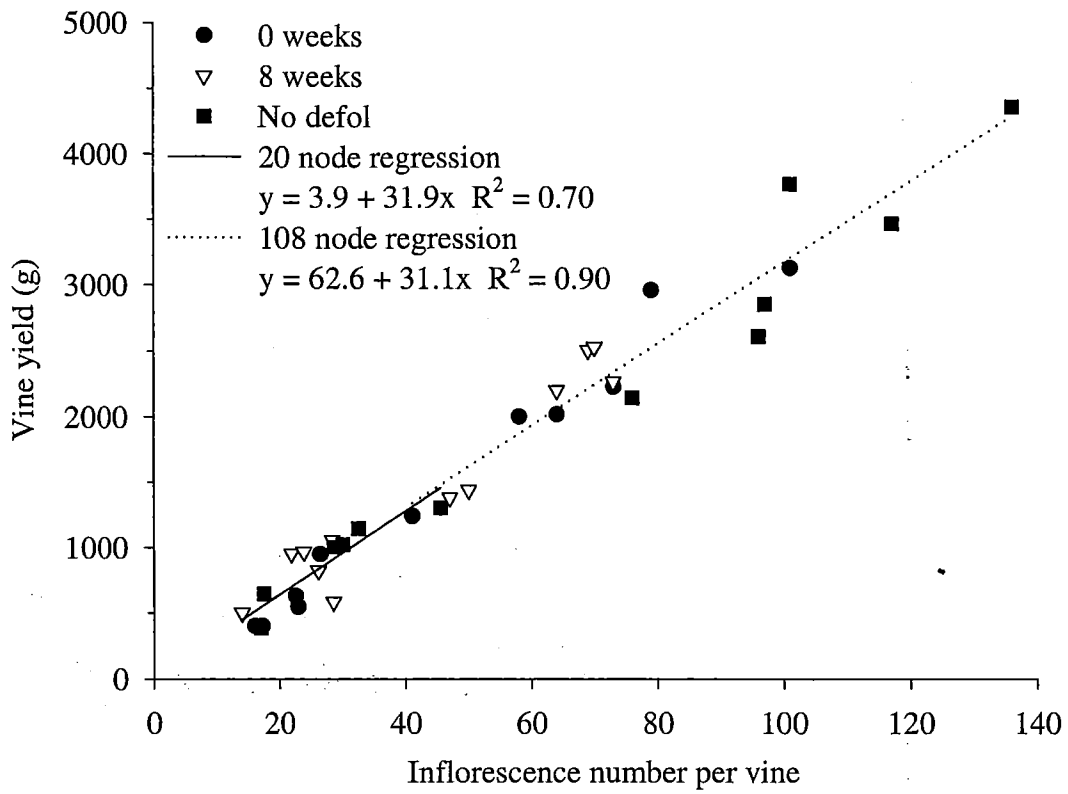

Figure 6.7 The linear relationship between inflorescence number per vine and vine yield (all vines, $y = 14.9 + 31.7x$ $R^2 = 0.96$).

Table 6.5 Simple and multiple (+) linear regression coefficients of determination (R^2), and probability values from the relationships between inflorescence number per vine and inflorescences per shoot and shoots per vine.

Node number per vine	**20 node vines**		**108 node vines**	
Variate	**R^2**	***P***	**R^2**	***P***
Inflorescence number per vine:				
Inflorescences per shoot	0.14	0.081	0.87	< 0.001
Shoots per vine	0.48	0.001	0.11	0.101
Inflorescences per shoot + shoots per vine	0.75	< 0.001	0.92	< 0.001

6.3.2.3 Relationship between carbohydrate reserves, sap flow and inflorescence number

To illustrate that the reduction in inflorescences per shoot was associated with vine CHO reserves and/or sap flow at the start of the growing season (bud burst) simple and multiple regressions between inflorescences per shoot and CHO and sap parameters were

performed. For 108 node vines linear relationships between root and trunk starch concentrations, sap flow and inflorescence number per shoot were found (Table 6.6). The coefficients of determination (R^2) indicated that between 50-65% of the variation in inflorescences per shoot was accounted for by root and trunk starch concentrations and sap flow at bud burst. Multiple linear regression of root and trunk concentrations + sap did not account for any more variation in inflorescences per shoot (Table 6.6). For 20 node vines no significant linear relationships could be found between root and trunk starch concentrations, sap flow and inflorescence number per shoot and (Table 6.6).

Table 6.6 Simple and multiple (+) linear regression coefficients of determination (R^2), and probability values from the relationship between CHO and sap parameters at bud burst (24/9/1999) and inflorescence number per shoot.

Node number per vine	**20 node vines**		**108 node vines**	
Variate	**R^2**	***P***	**R^2**	***P***
Inflorescence number per shoot:				
Root starch	0.00	0.432	0.55	< 0.001
Trunk starch	0.12	0.096	0.64	< 0.001
Sap Volume	0.04	0.226	0.47	0.001
Root starch + Sap Volume	0.05	0.379	0.52	0.002
Trunk starch + Sap Volume	0.06	0.252	0.63	< 0.001

6.3.2.4 Vegetative growth

Both early (0 weeks) and late (8 weeks) defoliation in the previous season reduced pruning weight by a third compared with no defoliation for both 20 and 108 node vines (Table 6.7). Shoot weight and diameter were reduced by both defoliations compared with no defoliation in 20 node vines, but in 108 node vines the reduction was not significant (Table 6.7). Defoliation had no effect on Ravaz index (yield to pruning weight ratio), but both early and late defoliations reduced vine capacity. Pruning weight per vine was not affected by the node number per vine, however, shoot weight on 108 node vines was less than a quarter of 20 node shoot weight (Table 6.7). The Ravaz index of 108 node vines was three fold higher than 20 node vines. The vine capacity of 108 node vines was higher than the vine capacity of 20 node vines (Table 6.7).

Table 6.7 The effect of defoliation at 0 and 8 weeks post-bloom in the previous season or not at all and node number per vine on pruning weight, Ravaz index and vine capacity (1999/2000).

Node number per vine	**20 nodes**			**108 nodes**			**Significance**		
Vine defoliation (weeks post bloom)	**0 weeks**	**8 weeks**	**No defol.**	**0 weeks**	**8 weeks**	**No defol.**	**[1]Nodes**	**[2]Defol.**	**[3]Interaction**
Pruning weight (g)	904 a[4]	914 a	1412 b	1020 a	931 a	1496 b	NS	**	NS
Shoot weight (g)	50.5 a	50.4 a	76.3 b	15.1 c	12.1 c	18.6 c	***	*	NS
Shoot diameter at 5th internode (mm)	5.6 a	6.0 a	7.4 b	4.8 c	4.6 c	5.0 ac	***	**	*
Ravaz index	0.7 a	0.9 a	0.7 a	2.3 b	2.3 b	2.4 b	***	NS	NS
Vine capacity (g)	660 a	710 ab	1010 bc	1130 c	1030 bc	1670 d	***	***	NS

[1], [2], & [3] main effects of Nodes, Defoliations and Interactions respectively at $P \leq 0.05$ (*), ≤ 0.01 (**), ≤ 0.001 (***) or not significant (NS).
[4]Means within the same row with the same letter are not significantly different at $P \leq 0.05$.

6.4 Discussion

6.4.1 Individual shoot leaf removal

6.4.1.1 Shoot carbohydrate accumulation

Despite the first shoot CHO sample not being taken until 31 days after the first leaf removal (0 weeks post-bloom) starch concentrations at the sixth internode of all shoots were very low, less than 1%Dwt (Figure 6.2b). Previous studies by Roper and Williams (1989) found that the starch concentrations of fully foliated Thompson Seedless shoots at one month post-bloom were as low as 0.3%Dwt, while Weaver and McCune (1959b) found starch concentrations of 2.5%Dwt in Thompson Seedless shoots (fully foliated) at just 14 days post-bloom. The starch concentrations presented in Figure 6.2b are consistent with the findings of Roper and Williams (1989). The low starch concentrations in both Chardonnay shoots (Figure 6.2b) and Thompson Seedless shoots (Roper and Williams 1989) suggest that at 31 days post-bloom shoots are not yet able to accumulate and store starch, presumably because so many other CHO sinks are present, namely shoot extension growth and berry growth.

From 31 days to 92 days after bloom, rapid increases in starch concentrations were observed in no leaf removal shoots, however the rate of starch accumulation in 0 week leaf removal shoots was lower (Figure 6.2b). Even though the rate of accumulation during this period was low, the modest increase in starch concentration that was observed possibly occurred as a result of the conversion of soluble sugar to starch. This is because no change in total CHO concentration was evident during the 31 to 92 day period after bloom (Figure 6.2c). Reductions in soluble sugar concentration from 31 to 92 days after bloom (Figure 6.2a) support this conclusion. The 8 week leaf removal treatment (just prior to véraison) completely halted the accumulation of starch from 63 days onwards (Figure 6.2b). Severely reduced photosynthate supply and the increased demand for CHO's by ripening berries at this time would have been responsible for the lack of starch accumulation in these shoots.

Starch accumulation in the shoots of no leaf removal ceased at 92 days after bloom, well before harvest and leaf fall. In contrast to this, Korkas *et al.* (1996a) found that starch continued to accumulate in Riesling shoots right up to the time of harvest. Although starch accumulation ceased before harvest in the Chardonnay shoots (Figure 6.2b) accumulation of total CHO did not cease until leaf fall (Figure 6.2c). Even though starch concentrations in the shoots of 0 week removal were lower than other treatments, starch accumulation albeit low continued right up to leaf fall (151 days after bloom), whereas in 8 week leaf removal and no leaf removal starch accumulation ceased at 63 (véraison) and 92 (mid-ripening) days after bloom respectively. Total CHO concentrations suggest that there was no net CHO reserve accumulation in the shoots of 0 week leaf removal until after 92 days post-bloom (Figure 6.2c). The source of CHO that contributed to this late accumulation remains unknown, but with no leaves present, it is possible that CHO may have been imported from trunk or root reserves. At bud burst soluble sugar, starch and total CHO concentrations were very similar to the concentrations at leaf fall (Figures 6.2a, b and c). Hence the effects of leaf removal at 0 and 8 weeks post-bloom in the previous on shoot CHO reserves persisted through to the following season.

6.4.1.2 Inflorescence initiation

According to single node measurements inflorescence number per shoot was progressively reduced at higher node positions the earlier cane leaf removal was performed in the previous season (Table 6.1, Figure 6.3). For example, no inflorescences were initiated at nodes 10, 11 and 12 on canes that had leaves removed at 0 weeks post-bloom in the previous season, while approximately 1.7 inflorescences per shoot were initiated at nodes 10, 11 and 12 on canes that had no leaf removal (Figure 6.3). The response of each node position to leaf removal at different times indicates that such treatments were negatively affecting the inflorescence initiation process in latent buds and also revealed that inflorescence initiation is a sequential process as previously shown by Buttrose (1969a, 1974a), Lavee *et al.* (1967) and Swanepoel and Archer (1988).

In Chenin blanc shoots Swanepoel and Archer (1988) were able to illustrate that the first signs of inflorescence initiation in the latent bud of node 1 occurred up to 13 days before the start of current bloom (Figure 6.8) and that fully differentiated inflorescence

primordia were present in nodes 1 and 2 at current full bloom. Results presented in Figure 6.3 show that inflorescence number per shoot was not reduced at node positions 1 and 2 for any leaf removal compared with no leaf removal (Figure 6.3). This suggests that for the majority of the canes inflorescence initiation at these node positions was completed before the first leaf removal treatment was imposed at bloom in the previous season. Such findings are consistent with the timing of inflorescence initiation shown by Swanepoel and Archer (1988) (Figure 6.8).

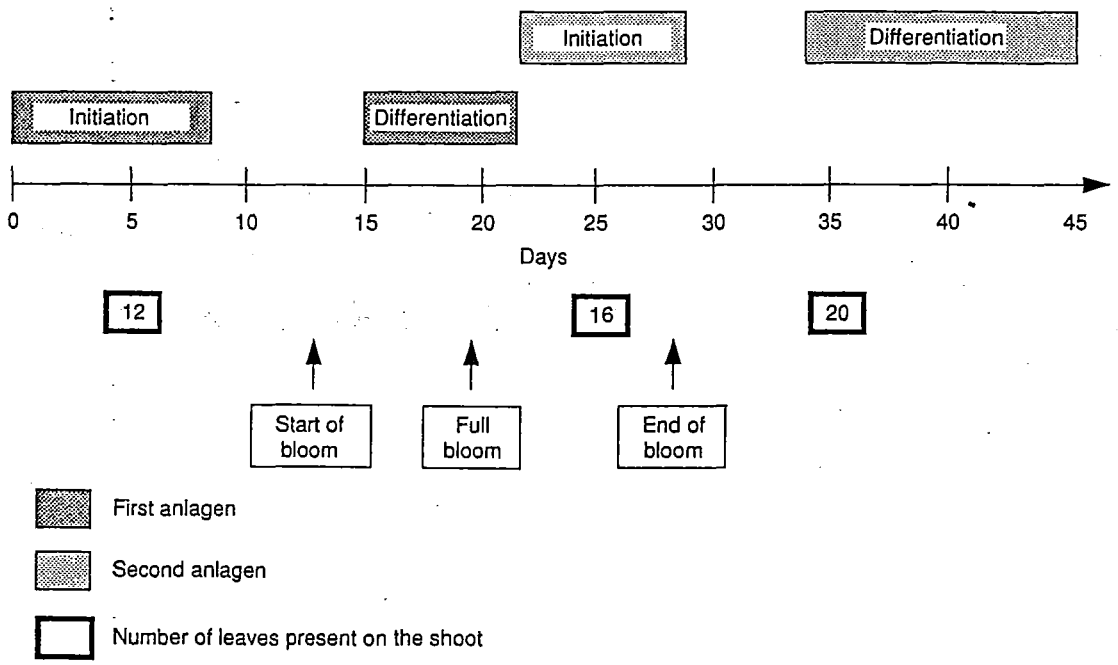

Figure 6.8 Diagrammatic representation of the events associated with the initiation of anlage and the differentiation of inflorescence primordia in a Chenin blanc bud (Swanepoel and Archer (1988).

Swanepoel and Archer (1988) further illustrated that the initiation of a second inflorescence primordia within a latent bud did not start until the first was differentiated (Figure 6.8). In relation to node position Swanepoel and Archer (1988) suggested that initiation at higher node positions later on (post-bloom period) may in fact be occurring at several node positions simultaneously. This was because Swanepoel and Archer (1988) found differentiating inflorescence primordia at several node positions at once, however they were unable to determine the timing of this simultaneous initiation activity. Given the Swanepoel and Archer (1988) observations, and the results presented here, a schematic diagram of the proposed sequential initiation of inflorescences is shown (Figure 6.9).

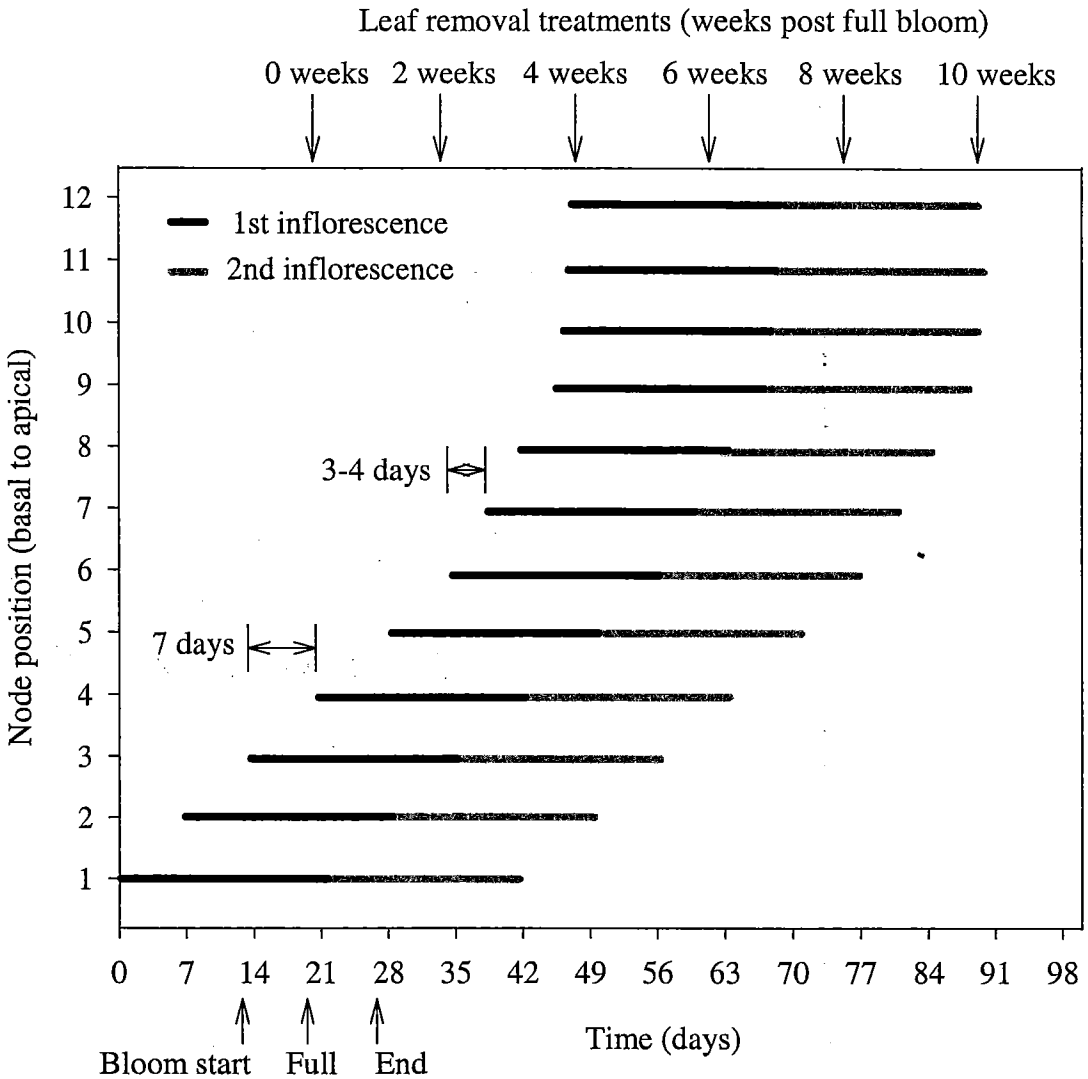

Figure 6.9 A schematic diagram of the proposed sequence of inflorescence inititation from basal to apical node positions over time.

The schematic diagram proposes that initially there is approximately a 7 day delay between the start of initiation at node 1 and the start of initiation in node 2. However as the weather warms and the rate of shoot node formation increases the delay is reduced to 3-4 days at about 18 days post full bloom and virtually no delay occurs from about 27 days post full bloom. The schematic diagram assumes, based on Swanepoel and Archer's (1988) data (Figure 6.8), that the time period for the initiation of two inflorescence primordia per node is consistently 40-45 days. If this assumption is correct, then significant overlap of inflorescence initiation between the first 12 node positions over time (as proposed in Figure 6.9) must have been occurring, especially when inflorescence results (Table 6.1, Figure 6.3) revealed that leaf removal from 10

weeks post-bloom in the previous season onwards had no effect on inflorescence number per shoot.

Before any further inferences are made about the inflorescence initiation process it is critical that the response of the canes on the vine (second group of six replicates) is examined in order to validate the single node cutting results as discussed above. In contrast to single node cuttings the reduction in inflorescences per cane on the vine was not the result of reduction in inflorescences per shoot but rather the result of reductions in percentage bud burst (hence shoot number) the earlier leaf removal was performed in the previous season (Table 6.1). The cause of reduced bud burst will be discussed in 6.4.1.3. Because bud burst was so low on early leaf removal canes an examination of possible node position effects with respect to inflorescences per shoot could not be made (too much missing data). As a consequence of this, the single node cutting results and the proposed sequence of inflorescence initiation could not be validated with any certainty. Therefore the proposed sequence of inflorescence initiation remains to be validated by further experimentation on vines growing in the vineyard.

Despite in situ cane vine assessment being unable to support the single node cutting results this does not mean the single node cutting results should be disregarded. The single node results and proposed sequence of inflorescence initiation imply that inflorescence initiation at each node is sensitive to the foliated status of the node. This suggests that the supply of photosynthates from the subtending leaf may play a pivotal role in latent bud development and the initiation of inflorescences within. The role of the subtending leaf in influencing inflorescence initiation is also supported by the work of Harnett (1993). Harnett (1993) performed alternate node defoliation on growing Chardonnay shoots and found that defoliated nodes had fewer inflorescences than non-defoliated nodes in the following season. The relationship shown between shoot starch concentrations at the end of the inflorescence initiation period and both inflorescence number per shoot and per cane (Figure 6.5) suggests that photosynthate supply is an important factor during the inflorescence initiation process. Carbon isotope studies by Hale and Weaver (1962) have illustrated that the subtending leaf is the major source of carbohydrates for latent bud development, while Botti and Sandoval (1990) have found that the accumulation of starch granules in the apex cells of latent buds was correlated

with the formation of inflorescence primordia. Based on the results presented here (Figures 6.3 and 6.5) and earlier studies there is strong evidence to conclude that carbohydrate supply from leaves may play just as important role as hormones (Srinivasan and Mullins 1981a) in initiation of inflorescence primordia. Physiologically this makes sense, as much energy is consumed during complex cell differentiation and therefore a good carbohydrate supply and plenty of starch granules in surrounding tissues would be required to sustain such a process. This conclusion is supported by similar studies in kiwifruit. Buwalda and Smith (1990) and Snelgar and Manson (1992) have found that previous season's leaf removal resulted in large reductions in flower numbers. They concluded that the large reduction in flower number indicated that flowering depended on the accumulation of a critical level of photosynthate rather than the reception of some floral stimulus at a discrete point in time. However, neither Buwalda and Smith (1990) or Snelgar and Manson (1992) were specific on where in the vine the accumulation of a critical level of photosynthate was important in terms of determining flower number in the following season.

6.4.1.3 Bud burst

Results shown in Figure 6.5 suggest that the cause of reduced bud burst was the low concentration of starch in shoots post-véraison (92 days post-bloom) in the previous season. Regression analysis of shoot starch concentrations at 92 days post-bloom against percentage bud burst revealed a quadratic response, that is, initial increases in starch concentration resulted in rapid increases in per cent bud burst, but further increases in starch concentration resulted in only small increases in per cent bud burst (Figure 6.5). However, no relationship between shoot starch concentrations at 151 and 276 days post-bloom (time of bud burst) and percentage bud burst could be found. Such results imply that shoot CHO status during the later stages of latent bud development (post-véraison) may have been linked to some conditioning process within buds that determined whether they would be able to burst in the following season. The nature of the conditioning process remains unknown, but inducement of bud necrosis, as a result of reduced photosynthate supply, may be one possible reason. However, such an explanation is negated by the fact that percentage bud burst of single node cuttings was high for all leaf removal treatments (data not shown). Although no statistical analysis of bud burst at

each node position for the canes on the vine could be done, examination of raw data indicated early leaf removal in the previous season reduced the number of shoots at higher node positions. A possible cause of the reduction of bud burst and hence shoot number at higher node positions may have been localised variations in starch concentrations along the cane. It is possible that cane starch concentrations progressively declined at higher node positions and that the ability to convert to soluble sugars for winter protection was lost, consequently winter freeze damage may have killed many of the buds and cane tissue at higher node positions on early leaf removal canes. Sommer *et al.* (2000) have illustrated that even in fully foliated Sultana canes starch concentration declined at higher node positions. Therefore variations in CHO concentrations within the cane could indeed be a plausible reason for the reduction in bud burst on individual canes in response to previous season's leaf removal.

6.4.2 Vine defoliation

6.4.2.1 Carbohydrate reserves and sap flow

The response of over-wintering CHO reserves to the timing of previous season's vine defoliation was not strictly linear as previously shown in Chapter 5. In contrast to the results in Chapter 5 (Figures 5.2 and 5.3), both early (0 weeks post-bloom) and late (8 weeks post-bloom) vine defoliation reduced the concentration of starch in trunks by approximately the same proportion (Table 6.3). In roots, the later defoliation treatment actually resulted in the lowest starch concentrations (Table 6.3). A significant reduction in leaf area per vine and therefore photosynthate production, rather than crop load would have been the cause of the reduction in CHO reserves. Vine yields, hence crop load, were largely unaffected during season of defoliation (Appendix 3). Like starch concentration, sap flow (volume) was reduced by previous season's vine defoliation (Table 6.3), consequently sap volume was strongly correlated with root starch concentration in a linear fashion (Figure 6.6). This relationship suggests that root starch concentration plays an important role in sap flow throughout the vine. The flow of sap is initiated by the conversion of starch to soluble sugars in roots (Nassar and Kliewer 1966). Such conversion changes the osmotic potential of root tissues so that soil water is drawn in. As more water is drawn in the pressure increases (as high as 500kPa, Scholander *et*

al. 1955) and the air spaces within the xylem vessels are filled with surging sap as it moves up the trunk to the canes (Sperry *et al.* 1987).

Other factors, may also influence sap flow in the spring, for example, starch conversion in the trunk, the activity of starch degrading enzymes such as α-amylase and hence the rate of starch conversion to soluble sugar, soil temperature, mineral nutrient absorption and the size of the root system (McArtney 1998, Togonidze 1985). While the mechanism(s) ultimately responsible for controlling sap flow have not been elicited here, the results clearly provide evidence of a significant link between root starch concentrations and sap flow. Furthermore the data indicate that the measurement of sap flow may provide a good estimate of the CHO status of grapevines at the start of the growing season.

Although no differences in sap soluble sugar concentrations were found in relation to defoliation (Table 6.4), McArtney (1998) has illustrated that the concentration of soluble sugar (glucose, fructose and *myo*-inositol) in the sap changes over time once a cane has been cut. McArtney (1998) hypothesises that changes in sugar concentrations are a reflection of the concentrations of sugar in the sap in canes, trunks and roots as the total xylem column from root to cane is flushed out.

6.4.2.2 Impact of carbohydrate reserves on yield forming processes

The effects of vine defoliation in the previous season on yield only became apparent on 108 node vines (Table 6.5). This suggests CHO reserve stress caused by defoliation in the previous season (see Table 6.3) only became evident when vines were further stressed by the retention of an excessive number of nodes. Vine yield was reduced by more than 1000g on CHO stressed 108 node vines compared with non CHO stressed 108 node vines. For 20 node vines such an effect was not significant (Table 6.4). In the absence of any major differences in cluster size (Table 6.4), the large reductions in yield were almost solely the result of reductions in inflorescence and hence cluster numbers per vine. This is clearly illustrated in Figure 6.7 where most of the variation in yield (90%) of 108 node vines was attributed to inflorescence number. Even for 20 node vines where treatment differences were not significant a linear relationship did exist with a

slope that was very similar to that of the 108 node relationship (Figure 6.7). Consequently the vast majority (96%) of the variation in yield per vine for the whole experiment was attributed to inflorescence numbers per vine (Figure 6.7).

The CHO stress effect observed on 108 node vines with respect to inflorescence number was a consequence of a reduction in inflorescences per shoot rather than reduced bud burst (Table 6.4). This was clearly illustrated by the regressions shown in Table 6.5. In 108 node vines the primary cause of a reduction in inflorescences per vine was a reduction in inflorescences per shoot. In contrast to 108 node vines, variation in shoot number per vine was the primary cause of changes in inflorescence number per vine for 20 node vines (Table 6.5). Clingeleffer (1984) has also reported that the retention of a high node number on Sultana vines, as a consequence of minimal pruning, resulted in a 30% reduction in bunches (inflorescences) per shoot compared with vines that had fewer nodes. However, no physiological explanation for the reduction in bunches per shoot was provided.

Results shown in Tables 6.3 and 6.4 suggest that there is a link between the level of CHO reserve stress at bud burst and the ability to develop initiated inflorescence primordia on vines with a high node number. To support this statement, simple and multiple linear regressions between root, trunk starch concentrations, sap flow at bud burst and inflorescences per shoot revealed significant relationships for 108 node vines, but not for 20 node vines (Table 6.6). Of the parameters regressed, trunk starch concentration in 108 node vines showed the strongest association (R^2 = 0.64). Sap volume, whether on its own or combined with trunk or root starch concentration, was unable to account for any more variation in inflorescences per shoot (Table 6.6). Therefore it appears that sap volume was unable to provide a better estimate of the availability and supply of CHO reserves to the development of inflorescences than just measurements of starch concentrations at bud burst. Although the regressions revealed significant relationships between starch concentrations and inflorescences per shoot on 108 node vines the coefficients of determination (between 0.55 and 0.64) indicated that there was still variation in inflorescences per shoot that was not accounted for by changes in starch concentrations or sap flow.

The association between starch concentrations and inflorescences per shoot suggests that CHO reserve stress coupled with excessive node number resulted in a reduced supply of CHO to each node and as a consequence of this, the ability of each node to develop a strong shoot with its full complement of initiated inflorescences was inhibited. Field observations during inflorescence counting (1-2 months post bud burst) indicated that some weakly growing shoots had dead inflorescences (particularly in the second/apical inflorescence position) attached that were no longer than 1-2cm in length. The number of dead inflorescences was not recorded and hence not included in the inflorescence results. It is possible that many inflorescences abscised before inflorescences were counted. In hindsight, inflorescence counting should have been performed twice, early on in the season and again at bloom time. This would have provided clear evidence whether inflorescence abscission had occurred. Despite this, such observations support the conclusion that CHO reserve stress coupled with excessive node number reduced the ability of node and hence shoot to successfully develop their inflorescence(s).

Yield responses illustrate that despite a significant reduction in inflorescences per shoot on 108 node CHO stressed vines, yield per vine was much higher than on 20 node vines (Table 6.4). Thus the five fold increase in node number retained, outweighed the concomitant reduction in inflorescences per shoot. Similar findings by Clingeleffer (1984) and Tafazoli (1977a) support these results. Clingeleffer (1984) showed that, even though the number of bunches per shoot was reduced, they did not negate the effect of more nodes per vine on increased yield. Tafazoli (1977a) also showed that more nodes per vine increased yield despite a reduction in average cluster weight.

6.4.2.3 Vegetative growth, vine balance and capacity

The reduced pruning weight of CHO stressed vines caused by defoliation in the previous season (both 20 and 108 node vines) was the result of a decline in shoot weight (Table 6.7) rather than shoot number per vine (Table 6.4). The small light shoots (12 - 19g) on 108 node vines (Table 6.7, Plate 6.5) clearly illustrate very low shoot vigour, this is further supported by the narrow shoot diameters (Table 6.7). Similar findings by Clingeleffer (1984) showed that minimal pruning (high node number per vine) resulted in lighter shoots that were narrower, had shorter internodes and lacked lateral growth.

According to Smart and Robinson (1991) vines with moderate manageable shoot vigour have a fresh weight of 30-50g at the end of the growing season. The shoot weights on all high node vines (12-20g) therefore indicated low shoot vigour, while those of 20 node CHO stressed vines were of moderate vigour (50g), but shoots from 20 node non CHO stressed vines were vigorous (76g, Table 6.7, Plate 6.6). The low shoot vigour observed supports the proposition that a reduction in inflorescences per shoot was the consequence of low shoot vigour, which in turn was the consequence of the combination of CHO reserve stress and an excessive number of nodes per vine. In other words, CHO reserves were spread too thinly on 108 node CHO reserve stressed vines.

The Ravaz index for both CHO stressed and non CHO stressed vines were the same (Table 6.7) indicating that both reproductive and vegetative growth were depressed by CHO stress similarly across defoliation treatments. However, 108 nodes per vine increased the ratio of fruit weight to pruning weight nearly three fold compared with 20 nodes (Table 6.9), indicating that 20 node vines were under cropped. Even with a fruit to pruning weight ratio of 2.3:1 108 node vines appeared to be under cropped. According to Smart and Robinson (1991) vines in good balance should have a ratio of 5:1. However, in Canterbury's cool climate environment the Chardonnay vines in this experiment, whether CHO reserve stressed or not, were unable to achieve this ratio. This is because Canterbury experiences a relatively cool summer climate with a growing period that is shorter than many viticultural regions in Australia, Europe and North America.

Carbohydrate reserve stress induced by previous season's defoliation impacted negatively on the vine capacity of both 20 and 108 node vines (Table 6.7). The vine capacity results, like those shown in Chapter 5 (Table 5.5, Figure 5.7) suggest that CHO reserves play an important role in vine productivity in a cool climate, where compensatory photosynthate production during the growing season is limited by cooler temperatures and a shorter growing period. Retention of more nodes per vine was also shown in an increase in vine capacity (Table 6.7). For example, maximum potential capacity was not achieved by the retention of only 20 nodes per vine nor was it achieved by CHO stressed 108 node vines.

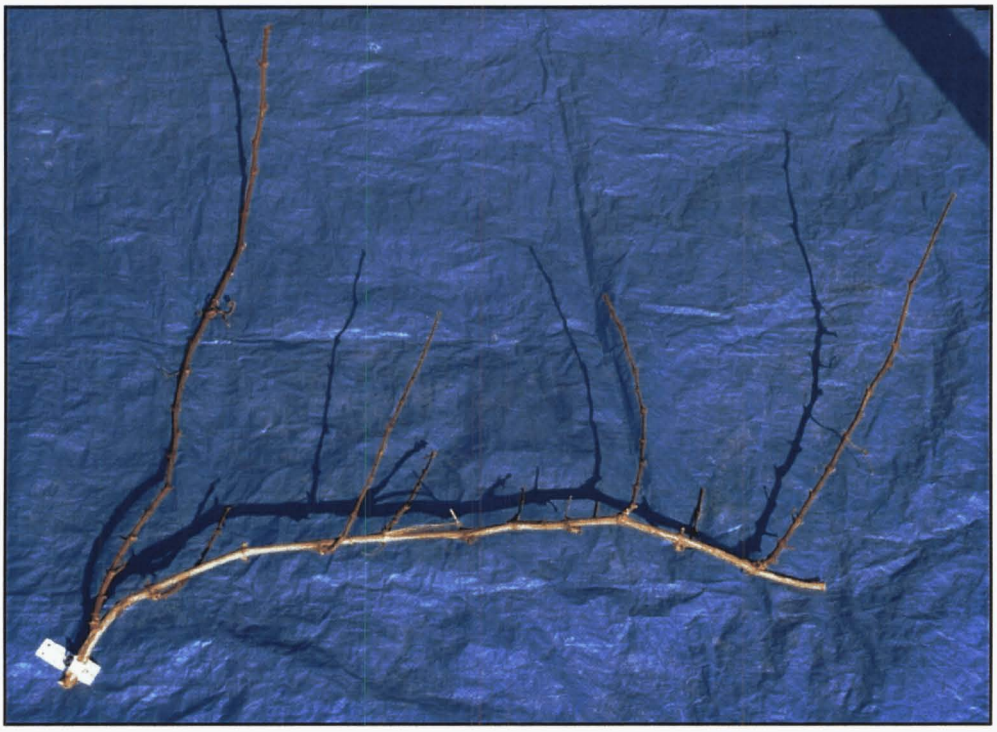

Plate 6.5 Low shoot vigour on CHO stressed 108 node vines.

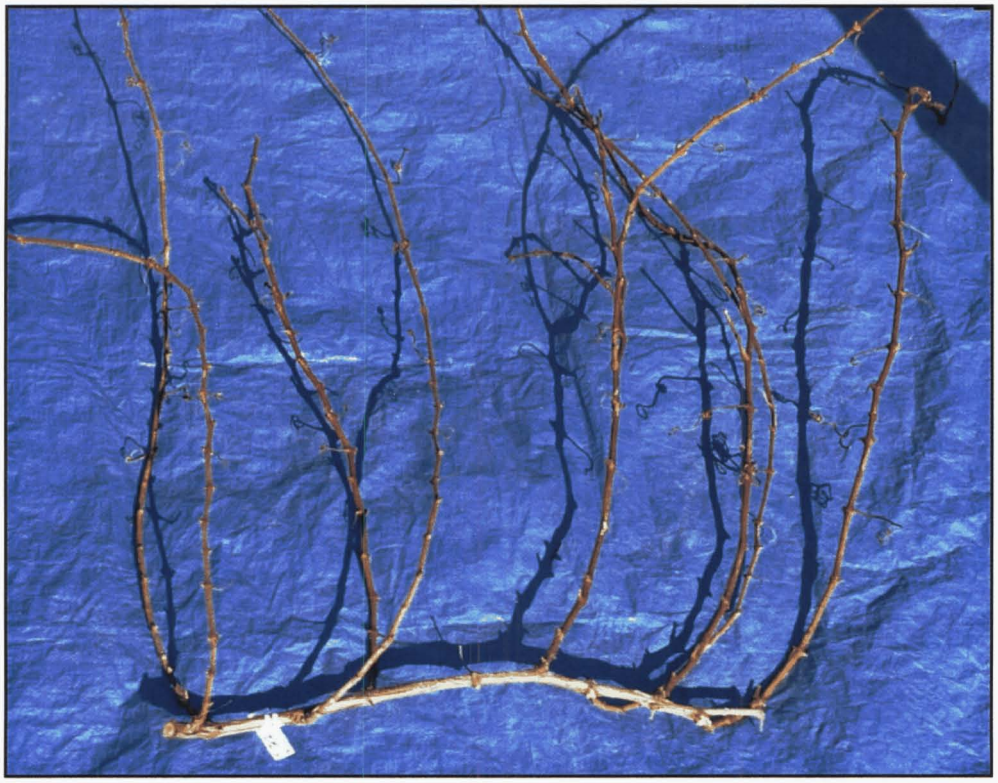

Plate 6.6 High shoot vigour on non CHO stressed 20 node vines.

6.5 Conclusions

A proposed scheme for the sequence of inflorescence initiation based on single node cutting results and earlier research suggests that approximately a 7 day delay occurs between the initiation of inflorescence primordia at node 1 and the initiation of primordia at node 2. As the rate of node formation increases (based on increasing temperatures) the delay is reduced to 3 to 4 days and virtually disappears approximately 1 month after current bloom. This proposed sequence of inflorescence initiation could not be validated by vine measurements because of the confounding effect of reduced bud burst on early leaf removal canes. The reduction in cane bud burst was associated with a reduction in the concentration of carbohydrates in canes during the later stages of latent bud development in the previous season. Inflorescence initiation results coupled with earlier research indicate that there is strong evidence to support a physiological link between the accumulation of shoot CHO's and the ability of nodes to initiate inflorescences and buds to burst in the following season. Furthermore inflorescence initiation results suggest that subtending leaves and the photosynthates they produce, play a pivotal role in latent bud development and inflorescence initiation within. The link between shoot CHO availability and the initiation of inflorescences could be further validated by imposing treatments like girdling and shoot topping (which are known to increase CHO availability) on vines during the initiation period. Experiments described in Chapter 7 will investigate whether or not enhanced shoot CHO availability is associated with increased inflorescence initiation.

The strong relationship shown between sap flow and root starch concentrations at bud burst suggests that sap flow is controlled by CHO reserves. Sap flow measurements at bud burst may therefore provide a good indication of the CHO status of vines at bud burst and thus allow for quicker and easier assessment of potential vine performance. Both trunk and root CHO reserve stress coupled with the retention of an excessive number of nodes per vine was shown to influence vine capacity by reducing shoot vigour and productivity. This was mediated by reductions in shoot diameter and weight and inflorescences per shoot respectively. Such results illustrate that the full complement of initiated inflorescence primordia per node may not develop into fruitful clusters when CHO reserve stress depresses shoot vigour. This however must be considered in relation

to the number of nodes retained per vine after winter pruning.

Cane and vine responses in this experiment show that leaf removal and defoliation affects both, but in different ways. In individual canes, leaf removal effects on inflorescence number were restricted to the treated cane. This provides evidence that canes are independent of each other and essentially act as "vines within vines" in response to stress situations like leaf removal. For vines, defoliation effects impacted on all growing shoots. The mechanisms by which cane and vine yields were reduced also differed. For canes reduced CHO's were linked to a decline in bud burst which in turn reduced shoot number and hence the number of inflorescences. For vines reduced CHO reserves were linked to a decline in inflorescences per shoot rather than bud burst. Thus the CHO status of localised (individual canes) as well as the whole vine (roots + trunk) strongly impacted on inflorescence number, the component of yield which had more influence on yield than any other measured in this experiment.

Chapter 7

The effect of trunk girdling and shoot topping on carbohydrate reserves and return bloom

7.1 Introduction

Results in Chapter 6 illustrated that a reduction in inflorescences per shoot in response to previous season's cane leaf removal was associated with reduced shoot CHO accumulation during the period of inflorescence initiation. Such results suggested that initiation of inflorescences, like shoot CHO reserves, was dependent on the supply of CHO from subtending leaves. Based on these observations, it is not unreasonable to suggest that girdling and/or shoot topping may increase shoot CHO accumulation, and therefore the supply of CHO to inflorescence initiation in latent buds, with the result that inflorescences per shoot (bud fertility) in the following season is increased. It is already well known that girdling and shoot topping increase the amount of photosynthates available for fruitset and berry growth and increase the concentrations of CHO's in leaves, stems and canes as early as 2 weeks after treatment (Caspari *et al.* 1998, Roper and Williams 1989, Weaver and McCune 1959b).

Girdling is an old viticultural practice and its use for improving fruitset and hastening maturity of grapes dates back to 1776 (Lambry 1817 cited in Lider and Sanderson 1959). Girdling is also extensively used to increase berry size in table grapes (Dabas *et al.* 1980, Lider and Sanderson 1959, Weaver 1976, Winkler 1953) and to a lesser extent is used to improve fruitset of shy yielding varieties of wine grapes (Chardonnay and

Gewürztraminer), and fruit maturity (Brown *et al.* 1988, Caspari *et al.* 1998, Coombe 1959, Dokoozlian *et al.* 1994, Jackson *et al.* 1984, Lider and Sanderson 1959, Tafazoli 1977b, Zabadal 1992). In a similar fashion to girdling, shoot topping at bloom time has been found to improve fruitset, berry weight, juice composition and yield (Coombe 1959, Jackson *et al.* 1984, Sharma and Jindal 1982, Skene 1969, Solari *et al.* 1988). Shoot topping is now an established practice and like girdling, shoot topping increases fruitset, berry size, and vine yield by disrupting the distribution of photosynthate throughout the vine in favour of fruit development.

Despite the benefits of girdling for fruit, it often comes at the expense of other aspects of vine growth in the season of girdling. Reports of reduced vine vitality have been noted, and these include:

- reduced vegetative growth and pruning weight (Bioletti and Flossfed 1918, Coombe 1959, Ezzili and Bejaoui 1998, Orth *et al.* 1989, Thomas and Barnard 1937b),
- a decrease in leaf photosynthesis (Harrell and Williams 1987, Hofacker 1978, Kriedeman and Lenz 1972, Roper and Williams 1989, Williams *et al.* 2000),
- impeded trunk growth (Lider and Sanderson 1959) and
- reduced root CHO reserves (Roper and Williams 1989,Weaver and McCune 1959b).

With so many 'side effects' noted above it is possible that any potential benefit to the initiation of inflorescences may be masked by possible reductions in over-wintering root CHO reserves, which in turn may reduce following season's floral and vegetative growth. This has been shown by defoliation experiments (Chapter 5 and 6), where reduced root and trunk CHO reserves were closely associated with reductions in flowering (inflorescence and flower number), yield and pruning weight. There is however, little literature to illustrate the impact of girdling or topping on CHO reserves and following season's vine growth and cropping. Therefore the primary aim of the experiment described in this chapter was to investigate whether increased shoot CHO accumulation as a consequence of shoot girdling and topping increased the number of inflorescences initiated per shoot. A secondly aim was to determine whether trunk girdling had detrimental effects on the accumulation of CHO reserves and whether this in turn impacted negatively on the flowering, yield and vegetative growth of grapevines in the following season.

7.2 Materials and Methods

7.2.1 Trunk girdling and shoot topping

A field trial was set up to investigate the effects of trunk girdling and whole vine shoot topping at bloom on carbohydrate reserve accumulation, inflorescence initiation and following season's flowering and yield performance. Mature VSP cane trained (two canes, ten nodes each) Chardonnay vines (own roots, same unnamed clone as used in Chapter 6) growing in the Lincoln University vineyard were selected for the experiment in early summer 1998. Trunk girdling and shoot topping treatments were combined in a 2 x 2 factorial design. Individual vines were girdled, topped, girdled and topped, or left alone (no girdle no top). Each treatment was replicated six times in a randomised block design. The treatments were imposed at 50% bloom (15th December 1998). In the case of the girdled vines a complete 4mm wide strip of cambium was removed from the mid region of the trunk (Plate 7.1). The topping treatment involved cutting the tops off every shoot per vine above the 14th node (Plate 7.2). Regrowth, in terms of lateral development, was not removed. Although not the primary focus of this experiment, measurement of flower number per inflorescence (using the methods described in Chapter 3.3), fruitset, cluster weight, vine yields and pruning weight during the girdling and topping season were recorded.

7.2.2 Shoot, trunk carbohydrate sampling and components of yield

The same shoot sample times as those chosen for the leaf removal experiment described in Chapter 6 (31, 63, 92, 151 and 276 days after 50% bloom) were used in this experiment. Sample shoots were chosen at random, but avoiding those that arose from spurs set aside for next season's canes. Shoots were cut off above the 5th node and the 6th internode region of the shoot was immediately frozen for CHO analysis in the laboratory. All shoot samples were analysed using the methods described in Chapter 3.1.

Plate 7.1 Fresh trunk girdle 4mm wide.

Plate 7.2 Shoots topped above the 14th node.

Trunk CHO samples were taken at 63 (pre-véraison) and 151 (leaf fall) days after treatment, both above and below the girdle zone region on all vines. At bud burst in the following season a trunk (below the girdle) and root sample were taken. Trunk CHO results at 151 days after treatment (leaf fall) indicated no positional effect (Table 7.2) hence only one core sample below the girdle was taken. Trunk samples were taken using the same coring device as described in Chapter 5.2.2. One root (1-1.5cm in diameter) per vine was sampled. All trunk and root CHO samples were analysed using the methods described in Chapter 3.1.

The components of yield measured in the season following girdling and topping treatment included the number of inflorescences formed per shoot (single node and vine), number of flowers formed per inflorescence, per cent fruitset, berries per cluster, cluster weight and vine yields. Flower number per inflorescence was determined using the methods described in Chapter 3.3. Shoot weight and diameter, and pruning weight, were also recorded at the end of the season.

7.2.3 Statistical analysis

All vine data from the 2 x 2 factorial experiment were analysed using general ANOVA using a Genstat statistical package (Genstat 5 Release 4.1. Copyright 1997, Lawes Agricultural Trust, Rothamsted Experimental Station). Mean separations were determined utilising least significant difference (lsd) at the 5% level of significance. Graphs were plotted using Sigma plot (Sigma plot for Windows version 4.01. Copyright 1986-1997 SPSS Inc.).

7.3 Results

7.3.1 Current season yield components

Trunk girdling at bloom time increased per cent fruitset (Table 7.1). Clusters on girdled vines had on average 47 more berries and were 57g heavier than clusters on non girdled vines. The proportion of seedless berries per cluster as well as mean seedless and seeded berry weight were also increased by girdling (Table 7.1). Cluster number per vine was unaffected by girdling, but as a consequence of heavier clusters vine yields were increased by as much as 1.5kg on girdled vines (Table 7.1). Fruit soluble solids at harvest were not affected by girdling, but vine pruning weight tended to be lower in girdled vines (Table 7.1). In contrast to girdling, shoot topping at bloom time had no effect on any yield components, vine yield or pruning weight (results not shown).

Table 7.1 The effect of trunk girdling at bloom time on per cent fruitset, berry number per cluster, berry and cluster weight, vine yields and fruit soluble solids at harvest, and pruning weight.

Treatment	Girdle	No girdle
Flower number per inflorescence	216 a[1]	199 a
Berry number per cluster	151 a	104 b
Per cent fruitset	70 a	53 b
Per cent seedless berries per cluster	48	25
Mean seedless berry weight (g)	0.44 a	0.22 a
Mean seeded berry weight (g)	1.72	1.31
Cluster weight (g)	169 a	112 b
Cluster number per vine	37 a	39 a
Vine yield (kg)	5.8 a	4.3 b
Fruit soluble solids (°brix)	19.9 a	20.6 a
Vine pruning weight (kg)	1.45 a	1.71 a

[1]Means within the same row with the same letter are not significantly different at $P \leq 0.05$

7.3.2 Shoot, trunk and root carbohydrate reserves

Shoot soluble sugar concentration was higher in girdled vines at 31 days after treatment compared with non-girdled vines (Figure 7.1a) and, even while sugar concentrations declined in the period 31 to 92 days after bloom, girdled vines continued to have higher shoot sugar concentration than non-girdled vines. By leaf fall the effects of girdling had gone and all shoots had a sugar concentration of 5-6%Dwt. Trunk girdling increased shoot starch concentrations at 31 days after treatment to 3%Dwt versus 1%Dwt for shoots on non-girdled vines (Figure 7.1b). However, the girdling effect had gone by 63 days after treatment when all shoots contained approximately 6%Dwt starch. Shoots on all vines continued to accumulate starch until about the time of harvest, when concentrations levelled out at 12%Dwt (Figure 7.1b). As a consequence of the girdling effect on sugar and starch concentrations, total CHO concentrations were also higher during the first 63 days after treatment, however by 92 days the girdling influence had gone (Figure 7.1c). Shoot topping had no effect on shoot soluble sugar (Figure 7.1a), starch (Figure 7.1b) or total CHO concentrations (Figure 7.1c).

At 63 days after girdling trunk soluble sugar concentration both above and below the girdled zone was higher than soluble sugar concentration in the trunks of non-girdled vines (Table 7.2). Starch concentration on the other hand was reduced below the girdled zone compared with non-girdled vines, but was increased above the girdle zone compared with non-girdled vines. Total CHO concentration responded in the same manner as starch (Table 7.2). By leaf fall (151 days after treatment) all girdling and position effects had gone. At bud burst (24/9/1999) of the season following treatment trunk soluble sugar concentrations were higher in girdled vines compared with those of non-girdled vines (Table 7.3), however starch and total CHO concentrations were not altered by girdling. In contrast to trunks, both root starch and total CHO concentrations were reduced by girdling (Table 7.3). Root soluble sugar concentrations were not affected by girdling. Shoot topping had no effect on over-wintering CHO reserves in either trunks or roots (Table 7.3).

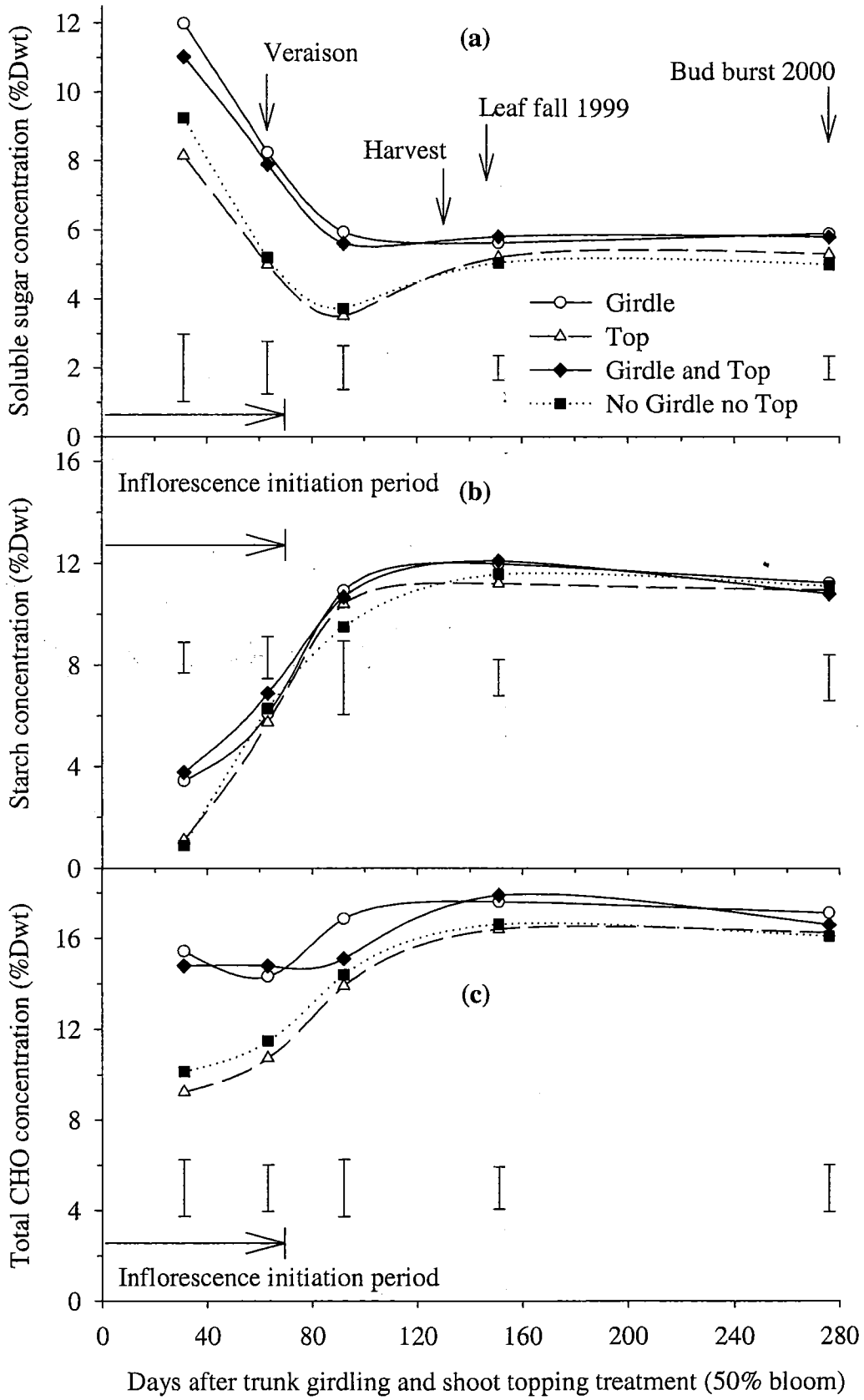

Figure 7.1 The effects of trunk girdling and shoot topping on the accumulation of (a) soluble sugar, (b) starch and (c) total CHO at the sixth internode of shoots. Bars represent lsd at 5% level of significance.

Table 7.2 The effect of trunk girdling on CHO concentrations above and below the girdle zone versus no trunk girdle at 63 days (pre-véraison) and 151days (leaf fall) after treatment.

Treatment	**Girdle**		**No Girdle**		**Significance**		
Position	**Above**	**Below**	**Above**	**Below**	**[1]Girdle**	**[2]Position**	**[3]Interaction**
63 days (pre-véraison)							
Soluble sugars (%Dwt)	1.9 a[4]	1.8 a	1.0 b	1.0 b	*	NS	NS
Starch (%Dwt)	13.9 a	10.1 b	12.7 c	13.0 c	NS	***	***
Total CHO (%Dwt)	15.8 a	11.9 b	13.7 c	14.0 c	NS	***	***
151 days (leaf fall)							
Soluble sugars (%Dwt)	10.1	10.6	10.2	10.8	NS	NS	NS
Starch (%Dwt)	8.7	8.8	8.6	8.8	NS	NS	NS
Total CHO (%Dwt)	18.8	19.3	18.7	19.6	NS	NS	NS

[1], [2], & [3] main effects of Girdle, Position and Interactions respectively at $P \leq 0.05$ (*), $P \leq 0.001$ (***) or not significant (NS).
[4]Means within the same row with the same letter are not significantly different at $P \leq 0.05$.

Table 7.3 The effect of trunk girdling and shoot topping in the previous season on the concentration of trunk and root CHO's at bud burst (24/9/1999, 276 days after treatment).

Treatment	Girdle		No Girdle		Significance		
	Top	No Top	Top	No Top	[1]Girdle	[2]Top	[3]Interaction
Trunk soluble sugars (%Dwt)	4.9 a[4]	4.6 a	3.1 b	3.4 b	*	NS	NS
Trunk starch (%Dwt)	9.5	9.2	10.2	10.6	NS	NS	NS
Trunk total CHO (%Dwt)	14.2	13.8	13.3	14.0	NS	NS	NS
Root soluble sugars (%Dwt)	3.8	4.0	3.8	3.6	NS	NS	NS
Root starch (%Dwt)	12.9 a	13.0 a	16.0 b	16.5 b	*	NS	NS
Root total CHO (%Dwt)	16.7 a	16.9 a	19.9 b	20.2 b	*	NS	NS

[1], [2], & [3] main effects of Girdle, Top and Interactions respectively at $P \leq 0.05$ (*) or not significant (NS).
[4]Means within the same row with the same letter are not significantly different at $P \leq 0.05$.

7.3.3 Return flowering and yields

Vines that were not girdled or topped in the previous season had a higher per cent bud burst and consequently a higher shoot number per vine than treated vines (Table 7.4). Inflorescence number per shoot (single node cuttings and on the vine) and inflorescence number per vine were not significantly affected by previous season's girdling or topping (Table 7.4). Girdling and topping treatments also had no significant effect on the number of initiated inflorescences per shoot for node positions 1 to 12 (Figure 7.2). Node position 1, irrespective of treatment, consistently had fewer inflorescences per shoot than higher node positions (Figure 7.2). Flower number per inflorescence was increased by the topping treatment irrespective of whether vines had also been girdled or not, inflorescences had on average 28 more flowers (15% more) per inflorescence than non-topped vines (Table 7.4). Girdling on the other hand had no effect on flower number per inflorescence. Percentage fruitset was consistent across all treatments and ranged between 25-27%. Berries per cluster and cluster weight were also higher on vines topped in the previous season compared with non-topped vines (Table 7.4). However, the heavier clusters on topped vines did not significantly alter vine yields compared with girdling or no treatment (Table 7.4).

7.3.4 Vegetative growth and vine balance

The pruning weight of girdled vines was reduced to 80% of non-girdled vine pruning weight (Table 7.5). Although not statistically significant the narrower, lighter shoots of girdled vines (Table 7.5) appeared to be the cause of reduced pruning. The ratios of yield to pruning weight (Ravaz index) were not altered by previous season's treatments, but they were low, ranging between 0.7 and 1.2 (Table 7.5). Vine capacity (an estimation of total annual above ground dry matter production) was reduced by vine girdling to 83% of the capacity of non-girdled vines.

Table 7.4 The effect of trunk girdling and shoot topping in the previous season on inflorescence and flower numbers, fruit parameters and vine yields.

Treatment	Girdle		No Girdle		Significance		
	Top	No Top	Top	No Top	[1]Girdle	[2]Top	[3]Interaction
Per cent bud burst	86 a[4]	79 a	82 a	97 b	NS	NS	**
Shoot number per vine	21 a	19 a	20 a	23 b	NS	NS	**
Inflorescence number per shoot (Single node)	1.63	1.55	1.63	1.27	NS	NS	NS
Inflorescence number per shoot (Vine)	1.45	1.47	1.71	1.37	NS	NS	NS
Inflorescence number per vine	30	28	33	32	NS	NS	NS
Flower number per inflorescence	194 a	159 b	175 a	154 b	NS	*	NS
Berry number per cluster	53 a	42 b	46 ab	40 b	NS	*	NS
Per cent fruitset	27	26	26	25	NS	NS	NS
Mean berry weight (g)	0.72	0.74	0.76	0.74	NS	NS	NS
Cluster weight (g)	44.0 a	35.7 b	39.2 ab	34.8 b	NS	*	NS
Yield per vine (g)	1340	1000	1300	1130	NS	NS	NS

[1], [2], & [3] main effects of Girdle, Top and Interactions respectively at $P \leq 0.05$ (*), ≤ 0.01 (**) or not significant (NS).
[4]Means within the same row with the same letter are not significantly different at $P \leq 0.05$.

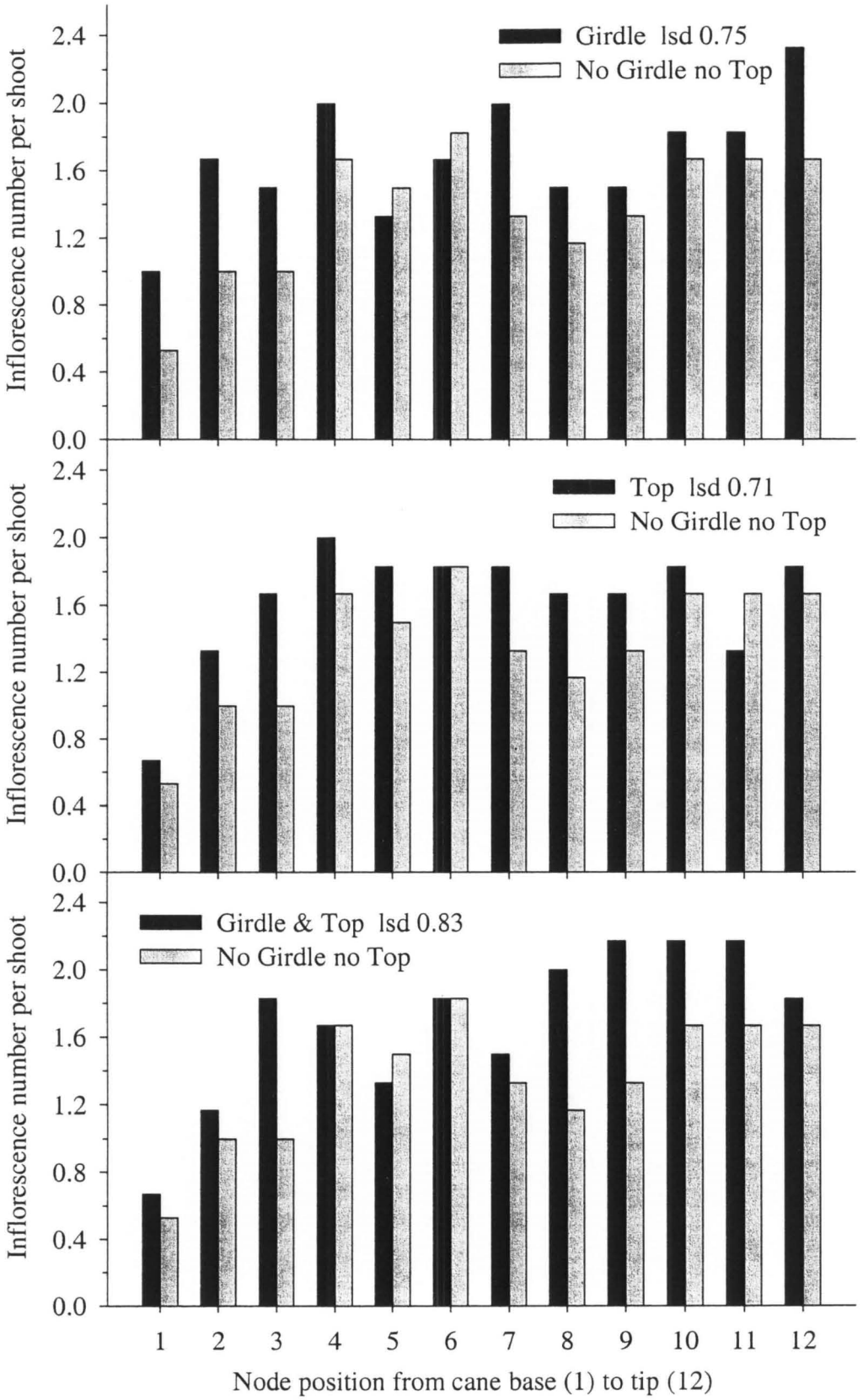

Figure 7.2 The effect of trunk girdling and shoot topping in the previous season on inflorescence number per shoot for node positions 1 to 12 (Single node cuttings) lsd at 5% level of significance.

Table 7.5 The effect of trunk girdling and shoot topping in the previous season on shoot and pruning weights, Ravaz index ratio and vine capacity.

Treatment	Girdle		No Girdle		Significance		
	Top	No Top	Top	No Top	[1]Girdle	[2]Top	[3]Interaction
Shoot weight (g)	61.5	65.7	79.4	71.1	NS	NS	NS
Shoot diameter (mm) 5th internode	6.8	6.8	7.6	7.9	NS	NS	NS
Pruning weight (g)	1250 a[4]	1250 a	1530 b	1650 b	*	NS	NS
Ravaz index ratio	1.1	0.9	0.9	0.7	NS	NS	NS
Vine capacity (g)	1020 a	940 a	1160 b	1190 b	*	NS	NS

[1], [2], & [3] main effects of Girdle, Top and Interactions respectively at $P \leq 0.05$ (*) or not significant (NS).
[4]Means within the same row with the same letter are not significantly different at $P \leq 0.05$.

7.4 Discussion

7.4.1 Current season yield components

Trunk girdling at 50% bloom increased per cent fruitset, which in turn resulted in more berries per cluster, heavier clusters and ultimately higher vine yields (Table 7.1). These findings are in agreement with previous girdling studies on both table and wine grapes (Brown *et al.* 1988, Caspari *et al.* 1998, Coombe 1959, Dokoozlian *et al.* 1994, Jackson *et al.* 1984, Lider and Sanderson 1959, Tafazoli 1977b, Zabadal 1992). The increase in the proportion of seedless berries per cluster and berry weight (Table 7.1) is also consistent with previous work by Brown *et al.* (1988) and Coombe (1959). However, in contrast to Coombe (1959) and Weaver and McCune (1959b), increases in vine crop load (yield) due to girdling did not depress the level of fruit soluble solids at harvest (Table 7.1). Coombe (1959) found that girdling of Sultana vines at bloom reduced the level of soluble solids in fruit compared with non girdled vines. Weaver and McCune (1959b) also found this, but in addition illustrated that, where girdles were kept open, fruit soluble solids were higher than those of non girdled vines. Although not statistically significant, girdling tended to reduced vine pruning weight (Table 7.1). This response is also consistent with previous work by Bioletti and Flossfeder (1918), Coombe 1959, Ezzili and Bejaoui (1998), Orth *et al.* (1989) and Thomas and Barnard (1937b). These authors found that trunk girdling reduced vegetative growth and hence pruning weight during the girdling season. The current season's yield results presented in this chapter (Table 7.1) have further illustrated that girdling increases fruit growth apical to girdle at the partial expense of vegetative growth.

7.4.2 Carbohydrate reserves

7.4.2.1 Shoots

While soluble sugar concentrations decreased in all shoots during the 31-92 day period following treatment, the difference between girdled and non-girdled treatments persisted, suggesting that girdling was not responsible for the reduction in sugar concentration. The reductions in soluble sugar concentrations were most probably the result of

conversion to starch, particularly so at 31 and 63 days after treatment, as no change in total CHO concentrations was evident between 31 and 63 days for girdled shoots (Figure 7.1c). Similar results in Chapter 6 (Figure 6.2) also illustrate that starch accumulation in shoots during the 31-63 day period was the consequence of the conversion of sugar to starch.

Regardless of the conversion process, shoot CHO results at 31 days after treatment indicated that girdling increased total CHO concentration compared with no girdling and that this increase was the result of both higher sugar and starch concentrations. Starch concentrations were, in fact, tripled by girdling at 31 days after treatment, from 1%Dwt in non-girdled vines to 3.5%Dwt in girdled vines (Figure 7.1b). Increased shoot starch concentrations as a result of girdling have been previously demonstrated by Roper and Williams (1989) and Weaver and McCune (1959b). At the same time period after girdling as in this trial, Roper and Williams (1989) found that girdling of Thompson Seedless vines increased starch concentration from 0.3%Dwt in the shoots of non-girdled vines to 1.2%Dwt in the shoots of girdled vines. In comparison with the Chardonnay shoot results presented in Figure 7.1b, Roper and Williams (1989) found that starch concentrations were considerably lower. This may be a reflection of a difference in grape variety. Weaver and McCune (1959b) found that the concentration of starch in the basal portions of Thompson Seedless shoots increased as soon as 2 weeks after treatment, from 2.5%Dwt in the shoots of non-girdled canes to 4%Dwt in the shoots of girdled canes. Roper and Williams (1989) who also used Thompson Seedless vines found, in contrast to Weaver and McCune (1959b), that their shoot starch concentrations were much lower suggesting differences may also be the consequence of other factors, for example, climate and crop load.

Post-véraison (approximately 92 days after treatment), the influence of girdling on shoot CHO reserves had gone (Figure 7.1). This was essentially a consequence of an increase of soluble sugar concentration in the shoots of non-girdled vines between 92 and 151 days after treatment (Figure 7.1a). Approaching winter dormancy results in an increase in shoot soluble sugar concentration (Winkler and Williams 1945) and thus explains the increase at this time. In contrast, the soluble sugar concentration in the shoots of girdled vines decreased until they reached the threshold level for winter dormancy (5-6%Dwt)

(Figure 7.1a). Therefore, in contrast to the effects of leaf removal (Chapter 6, Figure 6.2), the magnitude and the amount of time the girdling effect persisted was much less. In Chapter 6 shoot leaf removal was shown to reduce shoot CHO concentrations by as much as 50% and such reductions persisted right through to bud burst of the following season (Figure 6.1), whereas girdling only increased total CHO shoot accumulation by approximately 30% for the first 63 days after treatment (Figure 7.1c). Weaver and McCune (1959b) also illustrated that increases in shoot CHO concentrations shortly after girdling did not persist until the end of the growing season. The modest increase in shoot CHO's may have been the consequence of a much greater demand for CHO by fruit clusters on girdled vines during the 31-92 day period after treatment. Data presented in Table 7.1 show that cluster weight and yields on girdled vines were considerably heavier than those on non-girdled vines during the treatment season.

7.4.2.2 Trunks and roots

At 63 days after treatment, signs of girdle healing in the form of a callus growth were evident (field observations), but the girdles were not completely healed. Measurements of trunk CHO reserves above and below the girdled zone on both girdled and non-girdled vines further illustrated that the healing was not complete. Starch, soluble sugar and total CHO concentrations were all significantly higher above the girdled zone, while starch and total CHO concentrations were lower below the girdled zone compared with the same trunk positions on non-girdled vines (Table 7.2). Research by Hunter and Ruffner (2001) showed that shoot girdling resulted in increased sucrose concentrations in shoot bark tissue above the girdle as late as berry ripeness stage. Their findings provide support for the observation (Table 7.2) of increased soluble sugar concentration in trunks above the girdle during the berry development/ripening stage. The experimental results presented in this study (Table 7.2) indicated that when the vascular phloem connection was severed more CHO accumulated above the girdled zone. This enhanced accumulation above the girdled zone was occurring at the expense of CHO accumulation below the girdled zone (Table 7.2). Roper and Williams (1989) were unable to find any effect of girdling on trunk CHO reserves 31 days after girdling, but it is unclear whether they sampled trunk CHO's from above or below the girdle.

For most vines the girdles took between 63 and 84 days to completely heal (fusion of callus tissue, Plate 7.3). To determine whether CHO reserves were subsequently distributed evenly after healing was complete a second trunk sample was taken at leaf fall (approximately 151 days after girdling). No difference in trunk CHO reserves could be found above or below the girdled zone at leaf fall (Table 7.2), indicating that the reduced accumulation of CHO reserves below the girdle at 63 days was rectified once the essential phloem connection had been re-established by the healing of the girdle. Evidence of continued CHO reserve accumulation in the trunks of all vines after 92 days was shown by small increases in total CHO concentrations (Table 7.2). The increase in total CHO's suggest that while leaves were still on the vine some photosynthate was being distributed to the trunks.

Even though the topping treatment had shown no effect on shoot CHO's a possible influence on over-wintering trunk and root CHO reserves had not yet been ruled out. Analysis of trunk and root CHO samples at bud burst revealed that topping had no effect on CHO reserves (Table 7.3). Therefore it becomes evident that a one-off shoot top at bloom of the previous season had no influence on the distribution of photosynthates to the CHO reserves of the shoots, trunks or roots. The effect of girdling, on the other hand, was still clearly evident at bud burst of the following season. The trunks of girdled vines had elevated soluble sugar concentrations, while roots had lower starch and total CHO concentrations compared with non-girdled vines (Table 7.3). Reduced root CHO reserves in response to girdling has also been shown by Roper and Williams (1989) and Weaver and McCune (1959b). At 31 days after girdling Roper and Williams (1989) found the roots of girdled vines had lower starch and total CHO concentrations. Weaver and McCune (1959b) also illustrated that girdling impeded the accumulation of starch in the roots of Thompson Seedless vines as soon as 3 weeks after treatment. However, such differences had gone by the start of the dormant season. The recovery of root CHO reserves shown by Weaver and McCune (1959b) by the start of the dormant season was attributed to quicker girdle healing (42 days) and the presence of post-harvest leaf area for at least 4 weeks in the warmer Californian climate. In contrast to Weaver and McCune (1959b), the results presented in Table 7.3 show that in Canterbury's cool climate environment the prevention of CHO distribution to roots in the first 63-84 days following trunk girdling resulted in lower over-wintering root CHO reserves. Therefore

the findings clearly illustrate that girdling had significant negative 'side effects' on root CHO reserves (Table 7.3), which unlike shoot CHO reserves (Figure 7.1), persisted through to the following season.

The significant increase in crop load (yield) on girdled vines (Table 7.1) may have also influenced over-wintering root CHO reserves. Previous work by Balasubrahmanyam *et al.* (1978) and Weaver and McCune (1960) has illustrated that increases in crop load can reduce CHO reserves. However, in the experiment presented here, any direct effect of increased crop load on CHO reserve accumulation in roots would have been at least partially negated by the fact that the girdles were open (unhealed) during the major crop development period, that is, during the first 7-9 weeks after bloom. Therefore any direct influence of crop load on CHO reserves would have only been possible after the girdles had healed, by which time crop load (yield) had been set and fruit was already beginning to ripen. The impact of reduced over-wintering root CHO reserves on return flowering and yield and vegetative growth will be discussed in 7.4.3 and 7.4.4 respectively.

Plate 7.3 Girdles completely healed between 63 and 84 days after treatment.

7.4.3 Return flowering and yields

7.4.3.1 Inflorescence initiation

Examination of node positions (using single node cuttings) indicated that neither girdling nor topping had any inhibitory or stimulatory effect on the number of inflorescences initiated per shoot at any node position (Figure 7.2). This is in contrast to individual shoot leaf removal in Chapter 6, where early shoot leaf removal during the initiation period resulted in reduced shoot CHO reserves, which in turn was associated with fewer inflorescences per shoot (Figure 6.4). Consequently, the results of the experiment described here do not support the proposition that increases in inflorescences per shoot are the result of the effects of girdling and topping on enhanced photosynthate supply to latent buds and shoot CHO accumulation. Even though an increase in shoot CHO accumulation was shown (Figure 7.1), the elevated level of CHO's, particularly of starch was small in absolute terms and did not persist for a long period of time. Therefore it is possible that the small and relatively short period of enhanced photosynthate supply to latent buds and shoot CHO accumulation was inadequate to increase inflorescence initiation activity. However, it seems more probable that a threshold level for the association between shoot CHO's and inflorescence initiation exits. Averaged data presented in Figure 7.3. shows that there is a distinct levelling off in the relationship between shoot starch concentration, shortly after the completion of inflorescence initiation, and the number of inflorescences per shoot (Single node cuttings). This suggests that shoots with a minimum threshold level of 10%Dwt starch is sufficient for a maximum number of inflorescences to be initiated regardless of whether vines have been girdled, topped or both. The lack of response of inflorescence number per shoot to girdling and topping treatments at any node position (Figure 7.2), in contrast to leaf removal, adds weight to the proposition that the subtending leaf is of significant importance to the initiation of inflorescences in latent buds.

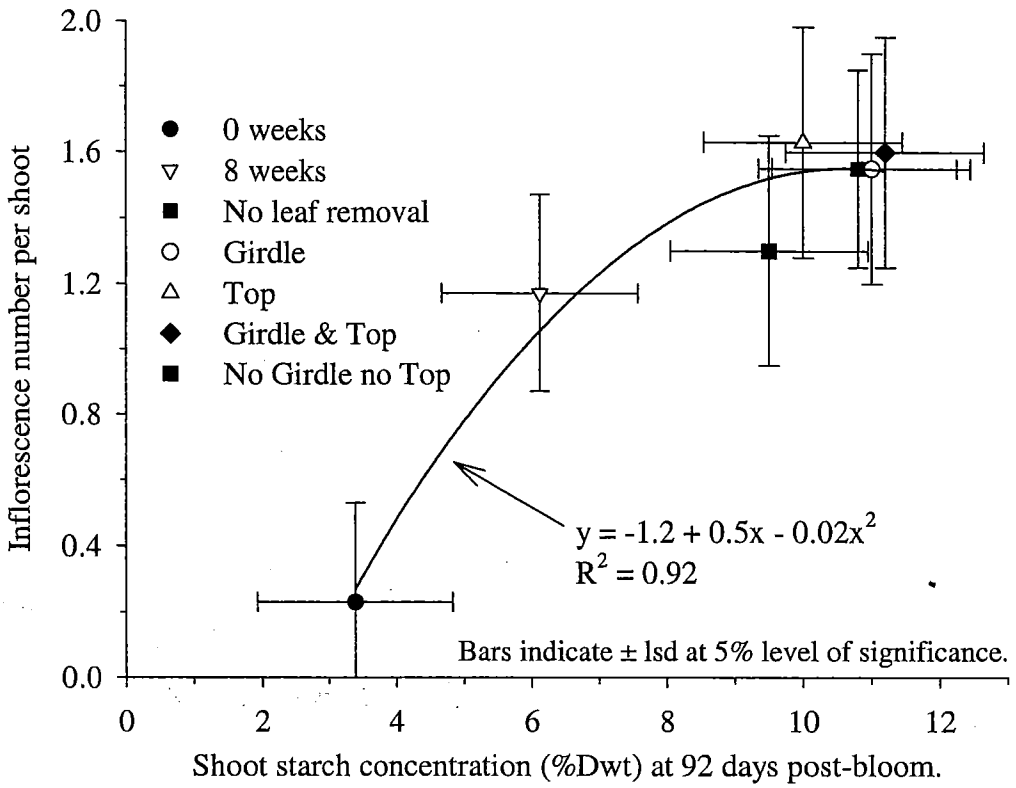

Figure 7.3 The relationship between shoot starch concentration at 92 days post-bloom and inflorescences per shoot in the following season across leaf removal and girdle top experiments (Single node cuttings).

7.4.3.2 Bud burst, inflorescence development and yields

Lower bud burst and shoot number on treated vines (Table 7.4) did not appear to be related to over-wintering CHO reserves in the shoots (canes), trunk or roots. This is because both topping and girdling in the previous season did not reduce shoot (cane) or trunk reserves (Figure 7.1, Table 7.3). Earlier work in Chapter 6 illustrated that there can be a strong relationship between shoot (cane) CHO reserves and bud burst, when CHO reserve accumulation is impeded by leaf removal. The small but significant reduction in root CHO reserves in girdled vines compared with topped or non-treated vines suggests that per cent bud burst was not dependent on root CHO reserves.

Inflorescence number per shoot (as measured on the vine) was not significantly affected by previous season's girdling or topping, a result that was consistent with single node measurements (Table 7.4). Consequently total inflorescence number per vine was not affected either, despite a small reduction in shoot number per vine in treated vines (Table

7.4). Previous work by Ezzili and Bejaoui (1998) has shown that girdling of Grenache noir grapevines in the previous season did not alter the number of inflorescences formed per shoot. However, in other vine crops such as kiwifruit, previous season's girdling has been reported to increase flower number (Davison 1980, Snelgar and Manson 1992). Yet, in other kiwifruit research, Currie (1997) found that girdling reduced flower number per vine by reducing either the number of lateral flowers per flower cluster or the number of floral shoots per vine. Such research findings illustrate in the case of kiwifruit that there is no consistent response of floral development to previous season's girdling. In grapes however, there is agreement between the results presented here (Table 7.4, Figure 7.2) and Ezzili and Bejaoui (1998) that girdling has no inhibitory or stimulatory effect on inflorescence number.

The number of flowers formed per inflorescence was not altered by the previous season's girdling. This is in contrast to the findings of Ezzili and Bejaoui (1998) who found that girdling increased the number of flowers on both basal and apical inflorescences by approximately 26%. Ezzili and Bejaoui (1998) suggested that girdling "disturbs" the content of ABA and probably of other similar compounds and that this disruption explains the increase in flower number. There is however at this stage no other literature to support this. In fact, the general consensus is that, of the plant hormones identified, exogenous applications of cytokinins and gibberellins have most influence on the formation of flowers (Srinivasan and Mullins 1981a). Alternatively the increases in flower number noted by Ezzili and Bejaoui (1998) could have been related to an increase in inflorescence primordia size induced by enhanced shoot CHO accumulation in response to girdling during the initiation of inflorescence primordia. However, because Ezzili and Bejaoui (1998) did not measure shoot CHO's, such a link cannot be established using their data.

The small, but significant, increase (15%) in flower number (Table 7.4) in response to topping in the previous season suggests that there must have been some lingering effect within the shoot (cane) or vine that stimulated more flowers to be formed. The increase in flower number does not appear to be related to CHO reserves in this instance as topping was shown to have no effect on shoot (cane), trunk or root CHO reserves at the time of flower formation (bud burst) (Figure 7.1, Table 7.3). It is possible that topping

may have disrupted the hormone balance (cytokinin vs. gibberellin) of the shoots during the initiation period in such a way as to increase inflorescence primordia size and hence flower number in the following season. However with no measurement being made of either hormones or primordia sizes this cannot be substantiated here.

With little variation in per cent fruitset, the small increase in flowers per inflorescence in topped vines resulted in more berries per cluster and subsequently heavier clusters (Table 7.4). Clusters had on average 17% more berries and were 15% heavier than clusters on non-topped vines. However, the small increase in cluster weight did not significantly alter vine yield (Table 7.4). This observation suggests that variation in other components of yield, for example, inflorescences per shoot, shoots per vine and consequently inflorescences (clusters) per vine (Table 7.4) masked the influence of more flowers per inflorescence hence berries per cluster on yield.

7.4.4 Vegetative growth and vine balance

The reduction in pruning weight (Table 7.4) appeared to be the consequence of a reduction in shoot vigour even though the analysis of shoot measurements did not reveal statistically significant differences. Reports of reduced vegetative growth and vine pruning weight in the season of girdling have been noted by (Bioletti and Flossfeder 1918, Ezzili and Bejaoui 1998, Orth *et al.* 1989, Thomas and Barnard 1937b, Coombe 1959), however, there have been no reports of reduced vegetative growth in the following season as shown by the results of this experiment (Table 7.4). Therefore these findings have illustrated that reductions in vegetative growth may also occur in the season following trunk girdling. Reduction in over-wintering root CHO reserves, in particular starch concentrations (Table 7.2) appear to be the most probable cause for the reduction in shoot vigour and pruning weight. Previous defoliation experiments in both Chapters 5 and 6 clearly illustrate that there were strong associations between the level of over-wintering root starch reserves at bud burst and subsequent shoot vigour (shoot weight and diameter) and pruning weight. Thus the results in this chapter provide further evidence to show that overall vine vegetative growth is reliant on the level of over-wintering CHO reserves present in the roots at bud burst and that there appears to be little capacity for compensatory vegetative growth in the following season.

7.5 Conclusions

The results of this experiment did not provide evidence to show that increased shoot CHO accumulation in response to girdling during the inflorescence initiation period was associated with an increase in inflorescence number per shoot. It is possible that the small and relatively short period of enhanced shoot CHO accumulation in girdled shoots was inadequate to increase inflorescence initiation activity. Additionally it is possible that a minimum starch concentration threshold of 10%Dwt in shoots (which was achieved by all treatments) was sufficient to support the successful initiation of a maximum number of inflorescences per node (shoot). Flower number per inflorescence was unaffected by girdling, but was increased by shoot topping. The small (15%) increase in flowers per inflorescence resulted in more berries per clusters and heavier clusters, however this did not significantly alter vine yields. The reason(s) for increased flowers per inflorescence in response to previous season's topping remain unclear in this experiment, but a possible alteration in the balance of hormones (cytokinin vs. gibberellin) or hormones vs. CHO may be responsible.

Carbohydrate results revealed that girdling had both temporary and longer term effects on CHO reserve accumulation in various parts of the vine. In trunks a temporary reduction in CHO accumulation was observed until such time as the girdles had healed. Similarly root CHO accumulation was reduced by trunk girdling, but persisted through to the following season. However, the small/moderate reduction in root CHO reserves in girdled vines did not impact on following season's vine flowering (no reduction in inflorescence or flower number was observed). In contrast vegetative growth and pruning weight, in the form of less vigorous shoots, was reduced by girdling.

The findings of this experiment illustrate that trunk girdling carried out for intended crop improvement (increased fruitset and berry size) in the current season may have a number of significant 'side effects' in the following season, these include; reduced root CHO reserves and vegetative growth. There is no indication that return bloom and yield is affected by trunk girdling or shoot topping. However reductions in flowering (inflorescence and/or flowers number) may occur, if the accumulation of over-wintering root CHO reserves is severely impeded by trunk girdling.

Chapter 8

General discussion and conclusions

The primary aims of the research presented in this thesis can be divided into two main areas. The first aim was to investigate the effects of treatments like moderate to severe leaf removal, trunk girdling and shoot topping along with shading and node number on the CHO physiology of grapevines growing in New Zealand's cool climate. Particular emphasis was placed on the impact of these treatments on the over-wintering CHO reserves in the trunks and roots of mature field grown Chardonnay grapevines. The second aim was to examine the influence of altered CHO physiology on flowering and fruiting processes of grapevines, in an attempt to better understand the role(s) carbohydrates play in the flowering and productivity of grapevines. This was determined by assessing the impact of over-wintering CHO reserves on subsequent flowering and also by investigating the influence of changes in photosynthate supply on the inflorescence initiation process. The general discussion and conclusions, will therefore focus on the relationship between vine CHO physiology and grapevine flowering (inflorescence initiation and development and flower formation).

8.1 Carbohydrate reserves

8.1.1 Shoots

The primary aim of Chapter 6 research was to investigate the influence of leaf removal on the supply of photosynthates to latent buds and the initiation of inflorescences within. This was measured by determining the concentrations of CHO in shoot tissue throughout

the growing season. Results demonstrated that leaf removal at either bloom or 8 weeks later, reduced shoot CHO concentrations by as much as 50% compared with no leaf removal (Figure 6.2). Such findings illustrate that the supply of photosynthates from leaves are of vital importance to the accumulation of CHO reserves in shoots and when removed, there is no other source of CHO to supply shoot reserves. This is because shoot reserves are relatively weak sinks for photosynthates compared with developing/ripening fruit clusters which are very strong sinks for photosynthates (Hale and Weaver 1962, Hunter and Visser 1988, Koblet 1969, Motomura 1990). Previous studies (Candolfi-Vasconcelos *et al.* 1994, Koblet *et al.* 1993, Koblet *et al.* 1996, Koblet *et al.* 1997) have shown that the sink strength of fruit clusters is so strong that when vines are severely photosynthate stressed (defoliation) during ripening stage CHO reserves in the trunks and roots of vines may be remobilised in order to mature fruit. Without the use of isotope carbon labelling it is not possible to demonstrate whether reductions in shoot CHO reserves were simply the result of reduced photosynthate supply or induced remobilisation to ripening fruit. However it seems the former was a more likely reason, as fruit soluble solids in defoliated vines were much lower than no leaf removal (Appendix 3), suggesting that no re-mobilisation activity from shoot reserves to fruit occurred.

In contrast to Chapter 6, the primary aim of the research detailed in Chapter 7 was to enhance the supply of photosynthates to latent buds and the initiation of inflorescence within by imposing shoot topping and trunk girdling treatments at bloom. As mentioned in Chapter 6, photosynthate supply was measured by determining the concentrations of CHO in shoot tissue throughout the growing season. The shoot apex at bloom time is usually the most active sink for photosynthates (Hale and Weaver 1962) and its removal (shoot topping) results in more photosynthate available for other processes such as fruitset (Coombe 1959). However, results in Chapter 7 established that shoot topping was unsuccessful in making more photosynthate available as no increase in shoot CHO's compared with non-topped shoots was observed (Figure 7.1). Girdling results on the other hand, showed that shoot CHO concentrations compared with no girdling after bloom were higher (Figure 7.1), but the increase was at most only 30% more than that of non-girdled vines and lasted for approximately 9 weeks. These findings are consistent with previous work (Roper and Williams 1989, Weaver and McCune 1959b), where

girdling was found to increase the availability of photosynthate for fruitset, berry growth and shoot CHO accumulation by preventing the phloem transport of photosynthates to trunk and root sinks. The relationship between changes in photosynthate supply, as measured by shoot CHO concentrations, and inflorescence initiation will be discussed in 8.2.

8.1.2 Trunks

Like earlier studies (Candolfi-Vasconcelos and Koblet 1990), previous season's defoliation was clearly shown to reduce over-wintering trunk CHO reserves as measured at bud burst (Chapters 5 and 6). The response to the timing of previous season's defoliation (Chapter 5) indicated that the longer the period of time with reduced leaf area (reduced supply of photosynthate) the lower the level of CHO reserves (Figure 5.3) However, results presented in Chapter 6 showed that early (at bloom) or late (8 weeks later) vine defoliation had similar effects on reducing over-wintering CHO reserves (Table 6.3). Such findings illustrate that even shorter periods of time without leaf area (defoliation at 8 weeks) can be just as inhibitory to accumulation of trunk CHO reserves as longer periods of time without leaf area (defoliation at 0 weeks). Further evidence of the effects of reduced photosynthate availability on trunk CHO reserve accumulation was shown by vine shading (Chapter 5) and trunk girdling (Chapter 7). Whole vine shading for as little as 5 weeks in the middle of the growing season (bloom-fruitset) reduced over-wintering trunk CHO reserves (Table 5.6). The reduction although statistically significant was not as large as that of vine defoliation (Chapter 5). Trunk girdling increased CHO reserve accumulation above the girdled zone, while it reduced accumulation below the girdle (Table 7.1). However these differences disappeared once girdles had healed later in the season, consequently over-wintering trunk CHO reserves were not altered by girdling in the previous season (Table 7.2).

8.1.3 Roots

Of all the reserves organs in the vine, roots were the most sensitive to defoliation, shading and girdling treatments (Chapters 5, 6 and 7). Like trunk reserves, the earlier defoliation was performed in the previous season the lower the level of over-wintering

root CHO reserves (Chapter 5). In fact the earliest defoliation treatment at 4 weeks post bloom reduced starch concentrations to approximately one tenth of that of non-defoliated vines (Figure 5.2). It is possible that such large reductions in root CHO reserve concentrations were the result of not only reduced photosynthate supply but also the remobilisation of root CHO reserves to maturing fruit during the season of defoliation. Previous studies by Candolfi-Vasconcelos *et al.* (1994), Koblet *et al.* (1993), Koblet *et al.* (1996) and Koblet *et al.* (1997) have illustrated that fruit sinks on severely photosynthate stressed vines (defoliation) can remobilise CHO reserves from trunks and roots to mature fruit.

In Chapter 6 results showed that both early (at bloom) and late (8 weeks post-bloom) vine defoliation reduced root CHO reserves, but unlike the effects of defoliation shown in Chapter 5 where starch concentrations were reduced to as little as a tenth of that on no defoliation, root starch reserves were only reduced to approximately a third of that of non-defoliated vines (Table 6.3). A number of reasons contribute to the difference in the magnitude of the reduction in root CHO reserves between the two defoliation experiments. For example the clone of Chardonnay (unknown) used in Chapter 6 research may have been more tolerant of defoliation stress and compensated better than the Mendoza clone of Chardonnay used in the experiment described in Chapter 5. Alternatively the presence of younger, more photosynthetically active leaves on foliated shoots (as part of the individual shoot leaf removal experiment) provided more photosynthate for CHO reserve accumulation than the older less photosynthetically active basal leaves retained on the shoots of the Mendoza vines used in Chapter 5 experiments. Candolfi-Vasconcelos and Koblet (1990) have shown that younger lateral leaves are more photosynthetically active than older main leaves. Another possible reason is that maturing fruit on the vines in Chapter 6 may not have initiated the remobilisation of root reserves during the season of defoliation. Measurements of berry soluble solids in the season of defoliation were markedly reduced by both early and late defoliation (Appendix 3), suggesting that CHO was not mobilised from other parts of the vine. In contrast, soluble solids of fruit on early defoliated vines (Chapter 5) was not reduced compared with later defoliations (Appendix 1), yet root starch concentrations were reduced to as little as a tenth of non-defoliated vines. Although any one, or a combination of the above reasons may explain the difference between the two defoliation

experiments, without the use of isotope carbon labelling to track photosynthate movement unequivocal evidence cannot be provided.

Further evidence of the sensitivity of roots is provided by vine shading (Chapter 5) and trunk girdling results (Chapter 7). Short term vine shading (5 weeks) resulted in small reductions in the concentration of over-wintering root CHO reserves (Table 5.6). Such response suggests the accumulation of root CHO was set back in time and could not be compensated for later in the season once the shading had been removed. Trunk girdling at bloom effectively cut the photosynthate supply to roots for approximately 9 weeks, consequently the accumulation of root CHO reserves, like shading, was set back in time. Previous research by Roper and Williams (1989) and Weaver and McCune (1959b) has also highlighted the sensitivity of root CHO reserves to girdling, but unlike the girdling results shown in Chapter 7, Weaver and McCune (1959b) illustrated that quicker girdle healing and post harvest photosynthesis compensated such that there was no over-wintering effects.

Significant reductions in CHO reserve concentrations (induced by defoliation, shading and trunk girdling; Figure 5.2, Tables 5.6 and 7.3) would have been the primary contributor to a reduction in CHO reserve content of the root system. However, it must also be considered that defoliation, shading and trunk girdling treatments may have also reduced root growth and hence the size of the root system during the season of treatment. Therefore a reduction in root system size may have also contributed to a change in the total CHO reserve content of the root system. Root growth in all experiments could not be investigated because treatment effects had to measured from season to season.

8.2 Yield components

The effects of defoliation, shading, and girdling on the relationship between carbohydrates and yield components can be divided into two areas; effects that occurred at or near the time of treatment and effects that occurred in the season following treatment. To illustrate this further, a flow diagram, based on the findings of this thesis, identifies the various steps (yield components) in the reproductive sequence of the grapevine that were affected by either photosynthate or reserve CHO supply (Figure 8.1).

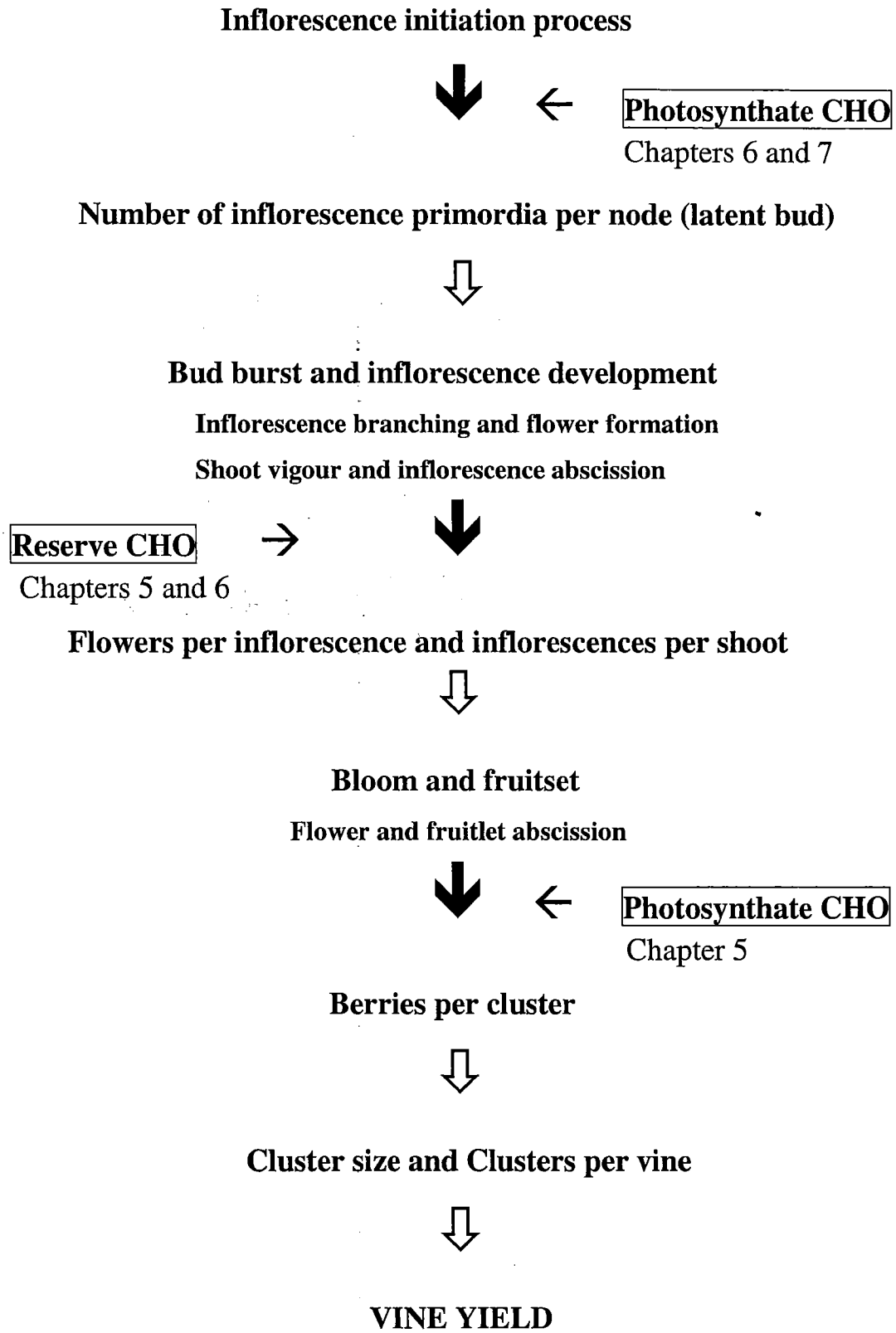

Figure 8.1 Steps (yield components) in the reproductive sequence of the grapevine which are influenced by carbohydrate physiology (photosynthate and reserves).

8.2.1 Inflorescence initiation

8.2.1.1 The sequence of initiation

Confirmation of the long sequential nature of inflorescence initiation, as previously demonstrated by (Buttrose 1969b, 1974a), Lavee *et al.* (1967) and Swanepoel and Archer (1988), was shown by individual shoot leaf removal (Chapter 6). Results provided evidence that inflorescence initiation in basal nodes of Chardonnay shoots commenced just before bloom, but was not complete at node 12 until approximately 8-10 weeks after bloom (Figure 6.3). These results, coupled with the earlier work of Swanepoel and Archer (1988), were used to develop a proposal for the precise nature of the sequential initiation of inflorescence primordia at each node position (Figure 6.9). Assuming that each node position has the capacity to initiate at least two inflorescence primordia, the initiation of the first primordium at node 1 is completed before the initiation of the second primordium at node 1 starts. However, initiation of the first primordium at node 2 starts approximately 7 days after the first primordium at node 1. The seven day delay between node positions reduces to 3-4 days at node 5 and virtually no delay occurs from about node 10 onwards. The reduction in delay is related to an increase in the rate of shoot growth and hence node formation as a consequence of increasing temperature as the peak of summer gets closer, particularly so in Canterbury's cool climate.

8.2.1.2 The role of carbohydrates

There is a growing body of previous research to suggest that carbohydrates play an important role in the initiation of inflorescences (Botti and Sandoval 1990, Lavee *et al.* 1967, May 1965, Sommer *et al.* 2000, Srinivasan and Mullins 1980a, Thomas and Barnard 1937a). Of this research strong evidence for the involvement of carbohydrates in the initiation of inflorescences is provided by Botti and Sandoval (1990) who illustrated that increased accumulation of starch granules in the apex cells of latent buds was correlated with more inflorescence primordia. Sommer *et al.* (2000) showed that decreases in starch concentration at higher node positions in a cane correlated with a decrease in inflorescences per shoot at these higher node positions. However, in contrast to the experiment described in Chapter 6, Sommer's *et al.* (2000) correlation was based

on shoot CHO samples that were measured well after the completion of inflorescence initiation. In Chapter 6 a strong association between shoot starch concentration immediately after the initiation period and inflorescences per shoot was illustrated (Figure 6.4). The quadratic nature of this relationship suggested that a threshold shoot starch concentration of 10-12%Dwt during this period was required for a maximum number of inflorescences per shoot (Figure 6.4). This conclusion was further supported by the relationship between starch concentration in girdled and topped shoots and the number of inflorescences per shoot (Figure 7.3). Such results imply the accumulation of starch in shoots was a good indicator of the supply of photosynthates to latent buds and the initiation of inflorescences within. Furthermore the study of individual node positions 1 to 12 (Chapter 6) showed sharp decreases in inflorescence number per shoot at each node position that was defoliated before inflorescence initiation was completed at that node (Figure 6.3). This in turn suggests that the subtending leaf of each node plays a pivotal role in the supply of CHO's to the latent buds and the initiation of inflorescences within. This conclusion is consistent with previous studies by Hale and Weaver (1962) and Smart *et al.* (1982). Carbon isotope work by Hale and Weaver (1962) illustrated the subtending leaf was the major source of CHO that accumulated in the latent bud. Smart *et al.* (1982) demonstrated a positive relationship between the illuminance of a leaf subtending a latent bud and the productivity of the shoot that arises from that latent bud in the following season. The initiation of inflorescences within the latent bud is probably mediated by the conversion of photosynthate from subtending leaves into starch granules in the apex cells of the latent buds as previously suggested by Botti and Sandoval (1990).

The relationship between carbohydrates and the initiation of inflorescences, however, cannot be considered in isolation as it is likely that other factors influenced this relationship, for example, hormones. Previous work (Srinivasan and Mullins (1981a) has illustrated that exogenous hormone applications (gibberellins and cytokinins) exert a significant influence on the initiation of inflorescences. Despite this, it is unclear whether the effects of exogenous hormones are in fact independent of carbohydrates, this is because exogenous hormone application (gibberellins and cytokinins) has also been shown to mobilise or 'attract' photosynthates either to, or away from, the site of application in grape shoots (Quinlan and Weaver 1969, 1970, Shindy 1967). Therefore it is possible that the observed effects of exogenous hormones on inflorescences per shoot

(Palma and Jackson 1989, Mullins 1968, Srinivasan and Mullins 1981a) are really the consequence of hormones mobilising or attracting photosynthates to the sites of inflorescence initiation. Whether endogenous hormones within the vine perform a similar function or not remains unclear at this time. Despite this uncertainty, the results presented in this thesis strongly indicate (regardless of how carbohydrates are transported to latent buds), that carbohydrates play just as an important role as hormones in determining the fruitfulness of latent buds and hence shoots in the following season (Figure 8.1).

Results from Chapter 5 provided some circumstantial evidence to suggest that initiation of inflorescences may also be indirectly affected by over-wintering CHO reserves. Shoots on highly CHO reserves stressed vines did not grow as fast as shoots on non CHO stressed vines during the earlier part of the season (Figure 5.7). These shoots were also less vigorous (narrower and lighter) and formed fewer inflorescences per node in the following season than shoots on non CHO stressed vines (Table 5.4, Table 5.7). It was concluded that less vigorous thinner shoots developed smaller latent buds, which in turn, initiated fewer inflorescences per node than larger latent buds on thicker more vigorous shoots.

8.2.2 Inflorescence development during and after bud burst

8.2.2.1 Inflorescences per shoot

Based on the results in Chapter 4 where more intensive vine defoliation was found to reduce inflorescence number per shoot in the following season, it was suggested that defoliation reduced inflorescence development in the following season by reducing the level of over-wintering CHO reserves available for new season's growth. Results presented in Chapter 5 initially supported this suggestion, however it became evident based on single node cutting data that defoliation was in fact affecting the initiation of inflorescences at, or shortly after the time of defoliation treatment. This conclusion was confirmed by individual shoot leaf removal results in Chapter 6. However, other results in Chapter 6 provided evidence that under certain circumstances the development of inflorescences after bud burst may be inhibited by the combination of CHO reserve stress

and a high node number vine. Vines that were both CHO reserve stressed at bud burst (Table 6.3) and had 108 nodes per vine produced fewer inflorescences per shoot than similarly CHO stressed vines that had only 20 nodes per vine (Table 6.4). The reduction in inflorescence number per shoot was shown to be the consequence of reduced shoot vigour (Table 6.7), which in turn was associated with an inadequate supply of CHO from reserves. Therefore these results indicated that the full complement of initiated inflorescence primordia within the latent bud may fail to develop properly and consequently die and abscise when shoots developed too weakly after bud burst. (Figure 8.1).

8.2.2.2 Flowers per inflorescence

Other aspects of inflorescence development after bud burst were also studied, namely inflorescence branching and individual flower development. Both the intensity and timing of previous season's defoliation resulted in substantial reductions in the number of flowers formed per inflorescence (Chapters 4 and 5). Results in Chapter 5 illustrated that at least part of the cause of reduced flower number was a reduction in the primary branching of the inflorescence (Table 5.1). A reduction in the number of flowers formed per branch was also identified as a cause of fewer flowers per inflorescence (Table 5.1). The reduction in flower number per branch would have been the result of a reduction in secondary and tertiary branching of the primary branches. This conclusion is supported by May (2000), who found that variation in flowers per inflorescence could not be related to primary branch number (because there was little difference in primary branch number), but were related to secondary and tertiary branching. Based on the fact that secondary and tertiary branching of the inflorescence and flower formation does not occur until bud burst (Barnard and Thomas 1933, Carolus 1970, May 2000, 1964) the results of defoliation experiments suggest that the levels of over-wintering CHO reserves in trunks and roots have a role in determining the number of flowers formed per inflorescence (Figure 5.6).

Further evidence of CHO reserves playing a role in determining flower number per inflorescence was demonstrated by the effects of vine shading (Figure 5.9) and the seasonal study (Figure 5.10). Vines used in the three season relationship (Figure 5.10)

were not defoliated or shaded, yet the trend for lower level of CHO reserves over consecutive seasons was met with corresponding reduction in flowers per inflorescence. Such findings illustrate a consistent positive relationship between CHO reserves and flowers per inflorescence and therefore further supports the premise that the determination of flowers per inflorescence during the bud burst period is dependent on the level of CHO reserves in roots and trunks. However, like inflorescence initiation, the dependency of flower number per inflorescence on CHO reserves cannot be considered in isolation from hormones. Previous work (Srinivasan and Mullins (1981a) has illustrated that exogenous hormone applications (gibberellins and cytokinins) have a significant effect on the subsequent number of flowers formed per inflorescence. Whether this is also the result of hormones regulating the supply of CHO reserves to developing inflorescence or not remains unclear at this time. The converse may also be possible, that is, where the level of CHO reserves are reduced the synthesis and/or transport of hormones to developing inflorescences is restricted. This may be facilitated by the effect that root CHO reserve concentrations have on sap flow (Figure 6.6) during bud burst.

In contrast to defoliation and shading experiments trunk girdling, did not alter flower number per inflorescence in the following season (Table 7.4) even though trunk girdling did result in a small, but significant, reduction in root CHO reserves (Table 7.3). Such findings, in the context of Chapter 5 results (where trunk CHO reserves were reduced), possibly suggest that trunk CHO reserves may be utilised in the determination of flowers per inflorescence before or in preference to root CHO reserves.

Shoot topping did not alter over-wintering trunk or root CHO reserves yet a small but significant increase in flower number was observed (Table 7.4). This contrasts with all previously published experiments. Furthermore there is no previous literature to show that topping induces such a response. Therefore one must be wary of whether these results are true and consistent effects of topping, given the relatively small (15%) increase in flower number. Proposed explanations for such effects can be provided. For example it was suggested (Chapter 7) that topping may have altered the hormone (cytokinin vs. gibberellin) or the hormone vs. carbohydrate balance of shoots during the inflorescence initiation phase, which may have stimulated an increase in primordia size

and thus the number of flowers formed in the following season. Earlier research shows that exogenous application of cytokinins can increase the number of flowers formed per inflorescence, while exogenous application of gibberellins can reduce flower number (Palma and Jackson 1989, Mullins 1968, Srinivasan and Mullins 1981a). There are however no studies to illustrate a link between endogenous levels of these hormones in vines at bud burst and the number of flowers formed per inflorescence. Therefore such research (Palma and Jackson 1989, Mullins 1968, Srinivasan and Mullins 1981) should be treated with caution in terms of the relationship between hormones and flower number determination.

8.3 Practical implications for vine management

In cool climate viticulture much has been made of the benefits of leaf removal on current season's fruit maturation and juice composition, and subsequent wine quality, but often little consideration is given to the effects leaf removal has on other vine processes. Similar comments can also be made about girdling and topping practices. One of the aims of the research presented in this thesis was to concentrate on the potential effects of these practices on other processes, in particular, vine CHO physiology in relation to vine flowering, yields and vegetative growth over successive seasons. Some of the defoliation treatments outlined in the experiments of Chapters 4, 5 and 6 were more severe than is typical of normal leaf removal practice. However they are representative of catastrophic events such as early frost and therefore bear some practical relevance to what can occur in the vineyard environment. One of the most important findings of these experiments was that vine defoliation from bloom through to approximately véraison was reducing both the number of inflorescence initiated, the accumulation of over-wintering CHO reserves in trunks and roots and the number of flowers formed per inflorescence. Furthermore defoliation was shown to affect individual node positions with respect to inflorescence initiation (Chapter 6). In the context of leaf removal (which is often performed soon after fruitset), such findings demonstrate that the number of inflorescences initiated could be reduced by such practices. Therefore it is recommended that leaf removal should be delayed until closer to véraison if the viticulturist wishes to avoid a reduction in inflorescences per shoot in the following season. A similar recommendation is suggested if the viticulturist wishes to avoid any reduction in over-

wintering CHO reserves in both trunks and roots and flowers per inflorescence in the following season. This is especially relevant in situations where there is no post harvest photosynthesis and CHO reserve accumulation.

Having noted the influence of defoliation, the seasonal variation results (Chapter 5) illustrate that large variations in CHO reserves from season to season can occur even when vines have not been defoliated. Such variation can be ultimately ascribed to environmental conditions particularly heat summation and sunshine hours and hence photosynthate production (Appendix 2). In below average seasons (where both heat summation and solar radiation are below optimum) the viticulturist may consider reducing crop load in order to allow sufficient photosynthate to be partitioned to reserves, bearing in mind that shoot, trunk and root reserves are weak sinks for photosynthate while maturing fruit is on the vine. However such action is probably only going to have limited benefit. This is because below average heat summation (Growing Degree Days) and solar radiation will have direct influence on the supply of photosynthates to latent buds and the initiating inflorescences therein. In such circumstances maintaining low density canopies so that every subtending leaf is well illuminated and hence will be photosynthesising is the best action to take.

The retention of high node number per vine after winter pruning was shown to exacerbate the effects of CHO reserve stress on shoot vigour and inflorescence number per shoot. Such responses illustrate that inadequate pruning, that is, leaving too many nodes on the vine, can result in a reduction in individual shoot productivity. This does not necessarily mean vine yield is affected because the increase in nodes (shoots) per vine often outweighs the concomitant reduction in individual shoot productivity. However too many shoots and clusters (high cropload) may have implications for crop maturity, fruit quality and even following season's vine performance.

Vine management practices such as girdling and shoot topping are used to improve fruitset, berry size, vine yield and fruit maturation, however the effect on other vine processes receives little consideration. Results from experiments referred to in Chapter 7 show that both girdling and shoot topping if performed at bloom/fruitset have little influence on following season's vine flowering and yield. Therefore, unlike leaf removal,

such practices can be performed to improve current season's productivity (Appendix 4) if so desired without undue effect on yield forming process such as inflorescence initiation and flower formation. However, there was evidence that girdling can reduce over-wintering CHO reserves in roots. In this case (outlined in Chapter 7), the reduction was small and did not impact on following season's vine flowering. If the girdles had been kept open (prevented from healing) larger reductions in CHO reserves may have occurred, which in turn could have easily had negative effects on following season's flowering and yields. Thus a fine balancing act by the viticulturist needs to be performed when using trunk girdling.

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Appendices

Appendix 1 The effect of time of vine defoliation on berry size, cluster weight, vine yield, juice soluble solids at harvest (1/5/1998) and pruning weight (Chapter 5, 1998).

Time of defoliation (weeks post bloom)	4 weeks	8 weeks	12 weeks	No defol.	Linear Sig.[1]
Mean berry diameter (mm)	12.0 a[2]	13.5 a	13.2 a	13.2 a	NS
Mean cluster weight (g)	45 a	46 a	50 a	48 a	NS
Clusters per vine	27 a	26 a	27 a	29 a	NS
Vine yield (kg)	1.28 a	1.22 a	1.35 a	1.40 a	*
Soluble solids (°brix)	19.5 ab	19.1 a	19.8 b	22.0 c	*
Pruning weight (kg)	1.16 a	1.32a	1.54 ab	1.81 b	**

[1]Linear significance at $P \leq 0.01$ (**), $P \leq 0.05$ or not significant (NS). [2]Means within the same row with the same letter are not significantly different at $P \leq 0.05$.

Appendix 2 Meteorological data for the five growing seasons from 1997 to 2001 at the Lincoln University research vineyard.

Season[1]	1996-1997	1997-1998	1998-1999	1999-2000	2000-2001	Long term average
GDD[2]	890	1172	991	783	894	924
Solar radiation (W/m^2)	3999	4309	3986	3890	4040	3679
Rainfall (mm)	345	170	268	354	246	380
Mean max. (°C)	18.8	21.8	19.6	18.6	19.8	19.8
Mean min. (°C)	9.4	9.7	10.3	9.2	8.6	9.0
Mean (°C)	13.7	15.4	14.6	13.5	14.1	14.4

[1] 1st October to 30th of April for each season.
[2] Growing degree days calculated using base temperature of 10°C.
Source Lincoln University weather station.

Appendix 3 The effect of time of vine defoliation on cluster weight, vine yield, juice soluble solids at harvest (27/4/1999) and pruning weight (Chapter 6, 1999).

Time of vine defoliation (weeks post bloom)	0 weeks	8 weeks	No defol.	[1]Linear Sig.	[1]Quad Sig.
Mean cluster weight (g)	75 a[2]	86 a	90 a	*	NS
Clusters per vine	44 a	40 a	44 a	NS	NS
Vine yield (kg)	3.3 a	3.5 ab	4.0 b	*	NS
Soluble solids (°brix)	17.1 a	15.9 a	20.0 b	*	*
Pruning weight (kg)	1.81 a	1.64 a	1.72 a	NS	NS

[1]Linear and quadratic significance at $P \leq 0.05$ (*) or not significant (NS). [2]Means within the same row with the same letter are not significantly different at $P \leq 0.05$.